·Review·

Bioactivities and Pharmaceutical Effects of 1-Aminocyclopropanecarboxylic Acid

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Abstracts: **Objective** The 1-aminocyclopropanecarboxylic acid (ACPC) is a natural micromolecule non-protein amino acid that exists only in plants. Despite the determination of its regulating effects on plants, several bioactivities and effects on animals or humans are still unclear. This review focuses the pharmaceutical effects，above all, the neuroprotective effects of ACPC on the cerebro- and cardiovascular system. **Methods** Two hundred and seventy nine studies were selected and identified from a total of 2457 references in Medline and Chemical Abstracts from 1957 to 2008. Only 38 articles on neuroprotective effects of ACPC from seven different countries qualified to be included in the analysis. **Results** ACPC has altogether following six general neuroprotective effects on the brain and nervous system: anti-neurotoxicity induced by NMDA, dynorphin-A, glutamate, and morphine; protection from cerebral neurological injury induced by ischemia; antidepressant and anxiolytic effects; anti-convulsion and -seizures and control of epilepsy; facilitation of spatial learning and memory; and the effect of reducing alcohol consumption. **Conclusion** ACPC has shown a variety of effects on plants and animals. The bioactivities and pharmaceutical effects on animals are of great significance to medical research and public health. Further clinical trials or epidemiological studies are needed to determine its effect in humans. Food intervention with ACPC-rich vegetables and fruits may be a suitable therapy for cerebro- and cardiovascular system diseases. Administration of trace exogenous ACPC could produce vegetables and fruits of rich endogenous ACPC.

Key words: 1-aminocyclopropanecarboxylic acid; bioactivities; cerebro- and cardiovascular; neurological protection; non-protein acid; pharmaceutical effects

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Introduction

The spectrum of the most common diseases and causes of death has fundamentally changed in the course of the last century. Instead of infectious diseases, nowadays there are primarily worldwide cerebro-vascular and cardiovascular diseases (circulatory system diseases), which form the biggest risk, and are at the top of the cause-of-death statistics. Two widely known examples are the strokes and heart attacks (cardiac infarction). In Germany alone, twelve million people between the ages of 35 and 65 have a high risk of stroke or a heart attack. Unfortunately, many of them are unaware of this high risk. According to the World Health Organization (WHO), more than 17 million people worldwide die each year of the consequences of strokes or heart attacks.

Stroke is one of the most common diseases which severely damages human health. A large number of investigations indicated that the mortality of stroke comes right after the mortality of cancer and myo-cardial infarction. Strokes can be divided into two major kinds, the ischemic and the hemorrhagic. About one third of patients with cerebral infarction is disabled with hemiplegia and aphasia, and totally depends on

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others (Haque and Nasreen, 2008). Taking care of these patients is becoming a social problem with the aging of the population.

Neuron death after ischemia is considered to be the main cause of the disabling. In consequence, the solution for the worst sequelae of cerebral infarction would be the protection of the nerve cells. Over the past decade, the study on the excitatory amino acid and its receptor was prevailing and some breakthroughs were made. It is proven that glutamate is not only the principle excitatory neurotransmitter in the brain, mediating the activity of the most excitatory synapses and influencing the development of synaptic plasticity, but also a potent endogenous toxin which may lead to the neuronal injury and even death in a variety of pathologic conditions. Glutamate's over interaction with its receptors (*N*-methyl-*D*-aspartate Receptor, NMDAR) has been proposed as a key step in the pathophysiology of neuronal death following ischemic stroke (Dirnagl *et al*, 1999; Manev *et al*, 1990). Therefore, using the effects of NMDAR antagonists as neuroprotective drugs to protect against brain damage in neurological disorders such as stroke is a new path to treat the cerebral nervous system diseases (Jia *et al*, 2006). However, these agents have psychotomimetic properties in humans and disturbance in breath and blood pressure and other side effects (Olney *et al*, 1991). Thus, looking for a safe and effective antagonist at NMDAR is currently becoming the pioneer research of cerebrovascular diseases. The 1-aminocyclopropane carboxylic acid (ACPC) is an effective, new-type glutamate-site antagonist at NMDAR.

What is ACPC?

ACPC is a natural micromolecule non-protein amino acid existing only in plants, or a non-animal-type amino acid. It is important to mention that there is no ACPC in our human body. ACPC is a very unusual amino acid to animals as compared to plants (Meyer, Schneider, and Elstner, 1992). ACPC is one of the most important products originally derived from plants, such as fruits and vegetables, especially in tomatoes, kiwis, and celeries.

According to its chemical structure (Fig. 1), ACPC is composed of the following three parts: the cyclo-propane ring, which easily opens and changes into polymer; the carboxylic group, which usually gets lost; and the amino group, which often changes into imine. Because of this very unstable chemical structure, the miraculous amino acid has many unique and excellent bioactivities, but its extraction is, nevertheless, complicated,

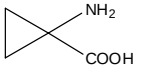


Fig. 1 Chemical structure of ACPC

and its synthesis is also quite complex. In most situations, no matter if using extracting or synthesis methods, a plastic compound or a glue, or very poor ACPC will be obtained. The problem is little yields and high cost. For example, only 254 mg of ACPC were derived from 120 L of pear juice (Burroughs, 1957).

The physiological importance of ACPC has aroused the study on various synthetic methods for this special amino acid. A good synthetic method with low cost but high yield has become a difficult problem worldwide. The scientists in the USA attempted to prepare ACPC with the zymotechnics, but up to now, the production has not started yet. However, in 1998, this hard nut in the world was at last cracked by Prof. ZHU Xu-xiang in Zhejiang Academy of Medical Sciences, China. He discovered a novel synthetic route to obtain ACPC and received a patent from China. The conditions of the reactions are mild, and the yield is good for mass production of ACPC with low cost. This is the first time that mass production of ACPC was realized in the world. In 2004, ZHU again discovered another new better synthetic route and received the patent. This method is also an environment-friendly preparation, which bids fair to be on stream in the commercial scale of ACPC.

History of ACPC

ACPC was first isolated and characterized from cider apples and perry pears by a British, Burroughs, in 1957 (Burroughs, 1957). Subsequently, ACPC was identified in ripe cowberries (Vaccinium, Vähätalo, and Virtanen, 1957). Later Burroughs (1960) observed that this amino acid increased considerably during storage of pears after harvest, and suggested that it might be related in some way to the process of fruit ripening, however, he was unable to assign a metabolic role for this unusual amino acid.

ACPC was nothing more than a non-protein amino acid which occurs in nature, and did not attract scientists' attention (Yang, 1998). Not until 1979, Adams and Yang had demonstrated its biosynthetic

pathway: "methionine → S-adenosylmethionine (SAM) → ACC → ethylene" (Adams and Yang, 1979). ACPC is the key intermediate in the conversion of methionine to ethylene. Through the work of Lürssen *et al* (1979), this route is well established.

In the same time period, Cameron *et al* (1979) identified that the synthesis of ACPC is the rate-limiting step in ethylene biosynthesis. Because the conversion of SAM to ACPC is mediated by the ACPC synthase (ACCS), which catalyzes the reaction, plays a very important role (Yu *et al*, 1979). However, the enzyme system which converts ACPC to ethylene had not yet been isolated and sufficiently characterized in the mean time. A few years later, the key enzyme of this section, which is the ethylene forming enzyme (EFE), was clarified by Yang and Hoffman (1984). Yang (1984) conjectured yet that the mechanism of the reaction from ACPC to ethylene was through the process of hydroxylation. Bousquet and Thimann (1984) discovered that the lipoxygenase system oxidizes ACPC to ethylene under physiological conditions. By now, the mechanism of ethylene biosynthesis has been thoroughly identified.

From an unknown object, ACPC has become the hot cake on the botany research field. Nowadays, researches of ACPC on plants have been fully-fledged. ACPC can promote the plant growth, produce a greater yield of crops and other plants, protect the plant against being encroached by viruses and sweeten fruits as well as regulate the germination, growth, blooming, fruiting, senescence and chlorophyll content and sex ratio of floral organs of plants.

As a non-protein amino acid, ACPC has its general character and multiple functions to intervene in the synthesis and catabolism of the protein amino acids and regulate and control the activity and property of common proteins. But more worth noting is its own characteristic and unique physiological property. After the 1980s, ACPC's medical and pharmaceutical effects were gradually discovered. Research on neuro-protection effect of ACPC is the longest and most complete among all studies of ACPC's effects. There are large numbers of evidence and documents to have reported this function. In comparison, research on ACPC's effects in other aspects has not yet reached maturity.

Principle of ACPC's anti-virus, disinfection, and disinsection

Amino acids are the basic structural building units of proteins. Plant and animal proteins generally include twenty "protein" amino acids used by cells in protein biosynthesis.They are encoded by the standard genetic code and are called proteinogenic or standard amino acids. In addition to the 20 "protein" amino acids universally distributed as protein constituents in living organisms, there are other amino acids of non-protein origin that can be found in food (Castro-Puyana *et al*, 2007). These other amino acids, which can either not be found in proteins (like carnitine, GABA, or L-DOPA) or are not encoded by the standard genetic code, are called non-protein amino acid (NPA) (Rubenstein, 2001). Hundreds types of NPAs have been found in nature and they have multiple functions in living organisms. Over 250 NPAs have been identified in plants (Swain, 1977). Most of the naturally occurring non-protein amino acids are produced in plants, such as ACPC, a small disubstituted cyclic amino acid.

A number of NPAs are intermediates in the synthesis and catabolism of the protein amino acids (Lea and Norris, 1976). Many of them are structurally similar to the components of common proteins. Under normal circumstances, NPAs are excluded from the process of protein synthesis in organism. Some NPAs, however, can be misincorporated into proteins (Butler and Peterson, 1967), which can occur due to the failure of the ribosomal protein synthesizing mechanisms to discriminate between the non-protein amino acid and protein amino acids. The incorporation of NPAs into proteins may be associated with autoimmune diseases in humans. Such misincorporation has been shown to occur in various species, including bacteria, in which impaired colony growth may ensue (Cowie and Cohen, 1957).

Therefore, non-protein amino acids may be used as anti-infective agents for treating an infection in a human or animal through inhibiting the growth of infective agents, which include bacterial infection comprising resistant strains of Acinetobacter, Klebsiella, Serratia, Staphylococcus aureus and Streptococcus pneumoniae, as well as vancomyocin-resistant enterococci, multi-drug resistant mycobacteria and other emerging resistant organisms; such as viral pathogens, fungi, Chlamydia, Mycoplasma, Rickettsia, yeast, helminthic parasites, and protozoans (Rubenstein, 2001). Therein, ACPC was certified of inhibiting growth of

Staphylococcus aureus and *Escherichia coli in vitro* to treat a *S. aureus* and an *E. coli* infection in a human or animal. The non-protein amino acids are suitable for use in the treatment of humans as well as for veterinary use treating diseases of animals, such as dogs, cows, chickens, birds, and so on. Furthermore, non-protein amino acids may be administered by a variety of routes including orally, parenterally, topically, by inhalation or implantation and optionally may be provided in pharmaceutically acceptable carries, for example a polymeric carrier (Rubenstein, 2001).

Theory of ACPC's neuroprotective effect

NMDAR is a complicated process. There are different sites on NMDAR involving glycine-site (Gly) and glutamate-site (Glu). Activities of NMDAR depend on the coordination of these sites, of which any single transmitter cannot open the ion channel. ACPC acts concurrently as a Gly-site partial agonist and a Glu-site competitive antagonist at NMDAR (Nahum-Levy *et al*, 1999), which has great significance to NMDAR (Fig. 2). This unique amino acid is both an agonist and an antagonist. Therefore, it can effectively inhibit glutamate induced excitatory toxicity mediated by NMDAR. ACPC acts as an agonist when glutamate is in physiological status, but acts as an antagonist when glutamate is in pathologic conditions, which is the exact beauty of ACPC.

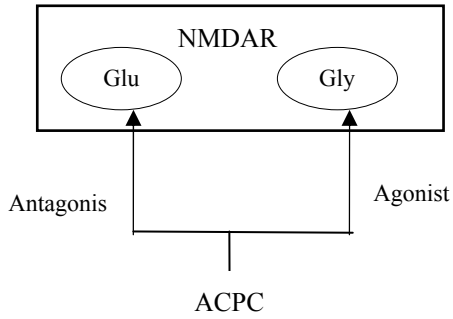


Fig. 2 Mechanism of ACPC to NMDAR

As generally known, glutamate is necessary to the excitability and plasticity of cerebral nerves, but it is also a potent endogenous toxin, if the nerves in the brain get overstimulated, which may result in neuronal injury, degeneration and even death (Manev *et al*, 1990). Overinteraction of glutamate receptors has been suggested to be the key reason of inducing neuronal death in the pathophysiology (Dirnagl *et al*, 1999). At the moment, ACPC will give antagonism towards the neurotoxicity caused by the high concentration of glutamate. It was reported that ACPC has the ability to attenuate glutamate-induced neurotoxicity under both *in vivo* and *in vitro* conditions (Layer, Bland, and Skolnick, 1993; Zhao *et al*, 2005). Excitotoxicity of glutamate includes two different expressions, namely the superexcitation of non-NMDAR mediated neuronal swelling with acute permeability, which could happen within a few hours, and the overexcitation of NMDAR mediated delayed neuronal injury, which could occur within several hours to a couple of days. NMDAR plays a very import role of mediating rapid and delayed neuronal death (DND) caused by cerebral ischemia, which is a significant pathological mechanism of neuronal death induced by ischemia (Fig. 3). As Papp Gruca, and Willner (2002) indicated that ACPC is not only a partial agonist at the strychnine- insensitive glycine receptor site on the NMDAR complex, but also a functional NMDA antagonist. Consequently, ACPC has neuroprotection acting through the agency of NMDAR.

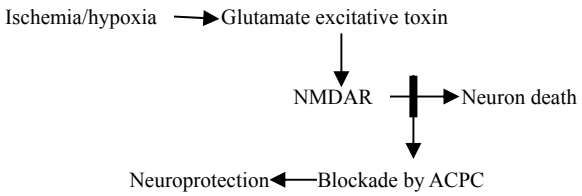


Fig. 3 Mechanism for the neuroprotection of ACPC

Aims of this subject

Currently, there is neither a single article nor literature of comprehensive systematic review about ACPC yet, which would introduce and sum up ACPC's excellent activities and functions, particularly, medical effects and the research status in the current world. To submit a paper of summary about this theme is important and necessary. It would be beneficial, if ACPC would get more popularity and attention in the research field so that more of its properties could be discovered. This work also offers the evidence of ACPC for the recent research.

The protective effectiveness of ACPC on the nervous system and stroke will be studied and estimated with literature review. In this case, the prevalence of sequelas induced by nerve cells impairment or death could be reduced or avoided by ACPC, if the results were positive. So that the patients might get well, for example, after stroke and the people's living standard after being ill would be greatly uplifted.

It was suggested that hypertension should be prevented and controlled with ACPC. It makes great sense in the current situation that the incidence of hypertension is very high, but there is no suitable medicine available for treatment that has no side-effects and dependency.

Finally, it is hoped to further promote the clinical research on ACPC and that ACPC will eventually be developed into a natural "medicament" without side effects. The product with the active substance of ACPC could be applied to clinical treatment and also included as a nutrient in everyday life diet. Then, in the near future, it should be successfully marketed all over the world for the benefit of human beings and reach an improvement of the health level of the population.

Results

There are large numbers of research evidence for the wide various effects of ACPC from different countries and years through many different kinds of animal tests. From altogether 279 selected articles, 38 articles report about the neuroprotections of ACPC and are used and analyzed in this chapter. Two articles studied its influences on plants and eleven studies researched the effects on animals as a whole. Two of them provided the evidence of ACPC's protection of kidneys against cell death. One article displayed finding that ACPC protected hepatocytes from hypoxic injury. Moreover, some literature investigated other functions of ACPC. One study reported the anti-virus or -bacteria and parasitic diseases activity, another one pointed out the action of anti-radiation, and a third article indicated the lipotropy effect of ACPC. Finally, some recent reports came out with its activities of cardiovascular system disorders such as controlling hypertension, prevention and treatment of thrombosis and stroke and so on.

Multi-effects of ACPC

Anti-virus/bacteria, disinfection, and disinsection on plants Functions and activities of ACPC were first known from research on plants. The beginning of research on ACPC owes a great deal to this source point. Overall, in the aspect of influence on plants, ACPC regulates many aspects of plant development and ripeness as the precursor of ethylene. Moreover, it can promote the plant growth, protect the plant against being encroached by virus and sweeten fruits as well as regulate the germination, blooming, fruiting, sense-cence and the chlorophyll content and sex ratio of floral organs of plants.

ACPC has significant effects on paddy rice against the rice sheath blight disease. Following three areas were set up for an experiment: an ACPC treatment area (1 g/kg water), a control area one (CK1, does not spray any agrochemical, negative control) and a control area two (CK2, sprays normal agrochemical to prevent diseases, positive control). Results revealed that the production of the ACPC area had increased by 16.7% compared to CK1, and decreased by 2.6% compared to CK2. But CK1 had a reduction in production with 16.5% compared to CK2. The situation of sheath blight disease was in the ACPC area with 28% infected leaf and uninfected stem, in CK2 with 23% infected leaf and also uninfected stem; nevertheless, in CK1 with above 50% infected leaf, 10% infected stem and 3% infected rice ear (Zhu, 1998). The results showed that ACPC was only slightly less effective than normal agrochemical (no significant difference), and it could be used instead of common agricultural chemicals.

ACPC is free from pollution, has no residue, and has no toxic side effects, which is of great significance to the pesticide residue in agriculture and the human food security. Thus, ACPC will be an effective agent on plant-beneficial as on anti-plant-pathogen interactions, and also will be protecting from disasters in agriculture.

Promoting the growth of animals Since 1980s, as the study of ACPC continued to deepen the plant field, ACPC's effects on animals were also gradually dis-covered and its pharmaceutical effects excited medical and pharmacological experts and increased their interests. ACPC is a partial agonist at the strychnine-insensitive glycine recognition site and meanwhile a glutamate-site functional antagonist on the NMDAR complex in the mammalian central nervous system with preclinical activity in animal models of neuroprotection and psychiatric illnesses. The pharmacokinetics of ACPC were studied in the rat, beagle dog, cynomolgus monkey, and human, and compared with data from literature about the mouse (Cherkofsky, 1995). ACPC acts through the agency of the NMDAR to affect the physiological action in animals. The main effects are such as promoting the growth of animals, the quality improve-ment of reproduction and the effects on the brain and

nervous systems. Furthermore, ACPC has also protection effects on kidney and liver as well as the cardiovascular system.

1. Increasing the rate of liveweight growth of fowl Testing data showed that ACPC can promote the growth of table poultry. The uncontrolled consecutive drinking of aqueous solution containing ACPC (1 mg/kg) for 10 d showed following effects on forty-days-old table poultry: significantly increase of weight on the market (the average weight in control group was 1415 g/feather, while in experimental group it was 1535 g/feather) and a decreased rate of weak poultry (control group with 8% compared to experimental group with 1.3%). Moreover, the table poultry that drank ACPC had a brightly-colored coat color and a rosy crest, which raised the commodity value (Ding *et al*, 2004).

2. Regulating the growth and development of silkworm As an additive, ACPC was sprayed on mulberry leaves or mixed in artificial feed (0.1–1 μg/100 g feed) to be fed to silkworms. The results indicated that the silkworms' weights increased by more than 30%; the growing development accelerated; the entire maturation process about shortened by one day and the cocoons' weights and rate increased so that a greater silk yield from these silkworms was produced. That explained, ACPC promotes the protein synthesis *in vivo* in silkworms (Zhang, Xu, and Zhu, 1990). Some would argue that this is related to ACPC, which is a strong and selective ligand at the glycine conditioning center on the NMDAR (Zhu and Xu, 1991).

Reproductive quality improvement

Increasing the laying rate of laying hens and the quality of eggs After primiparous hens drank ACPC 1 mg/kg water for one week, the laying rate increased significantly with 12.3% higher than the laying rate in the control group. Already two weeks ahead of schedule, the primiparous hens reached laying peaks as well as the platform of peak. The laying rate increased in the same period by more than 10%. At the same time, the weight of the eggs increased by 8% compared to the eggs from the control group. Eggs' percent of pass in the whole term was of a significant increase with 20%. Also the eggshell quality and appearance significantly improved, the eggs were smooth, delicate and rosy. This case showed that ACPC had a certain effect of increasing the weight and the quantity of ovum (Zhu, 2003).

Improving male reproductivity Nagasawa (1995) fed fifty-days-old male mice for two months with ACPC dissolved in tap water to the concentration of 0.001%. Two months later, four female mice were placed with each male for five consecutive nights (17:00–9:00). The male mice were killed after further 20 d of treatments. The results showed that the parturition rate of the female mice matched the feeding of the ACPC group (experimental group). The number of male mice had increased, while the rate of stillbirth and the stillborn pup had dropped significantly. But the differences between body weight, feed intake, water intake, endocrine organ weight, sperm motility, serum testosterone level and the litter size and pup growth in a same brood of the male mice in the control group (tap water) and experimental group were not significant (Nagasawa, 1995). These findings suggested that ACPC could improve male reproductive quality; however, the exact mechanism remains to be further studied.

According to these positive effects of ACPC on poultry and livestock, the development of ACPC to be a new-type feed additive is warranted. Firstly, the total dose of ACPC in a lifetime is only 1.0 mg/kg (this dose divided into 7–10 d to feed, sc 0.14–0.1 mg/kg·d) and equals to 0.5–1.0 kg of feeding fresh fodder containing a dose of ACPC, or the human body's daily dose of ACPC intake from fruits and vegetables. On the face of it, the usage amount of ACPC is very small. Secondly, the structure of ACPC is conclusive, the property is stable and the content is definite (purity > 99%), which is no distinction to natural ACPC found in plants. Thirdly, according to safety evaluation of ACPC, which consisted of six experiments as follows: Ⅰ acute toxicity test, Ⅱ subchronic toxicity test, Ⅲ mouse micronucleus test, Ⅳ mouse teratosperm test, Ⅴ Ames test, and Ⅵ conventional teratogenicity test, all test results were negative (–) and didn't find any toxic side effects. Finally, on the evidence of the test for residual quantity of ACPC in mouse urine by Howell *et al* (1995), which after orally dosing mice with ACPC (300 mg/kg), respectively 46% in the 0–24 h and 10% of the dose was excreted unchanged in the 24–48 h urines; and in livestock body by Yao *et al* (2001) that after three days fed with ACPC, no more dose could be detected *in vivo* (sensitivity 5

ppb). It proved that there were no residues of ACPC in the animal body.

Anti-virus/bacteria and parasitic diseases on animals It is a new anti-infection agent. Under normal circumstances, non-protein amino acid ACPC doesn't participate in the protein synthesis of the host, but can optionally sneak into the protein of the guest — the infection agent, selectively interferes with its anabolism, and will not influence the host. ACPC protects the host against bacteria of multi-resistance, especially *E. coli*, *S. aureus*, *C. albicans*, and several viruses such as NDV-LaSota (like H5N1), Newcastle Disease Virus, Influenza A and B, Parainfluenza, Coronavirus and Hepatitis A, B, C, D, and E (Zhu, 2005). Nowadays, in the situation that antibiotics is abused and the resistance is increasingly serious, thus, there is a need for the development of compounds which are useful as anti-infective agents with the fewest and mildest side effects on the host. ACPC, as the novel antibiotics of new mechanisms, is of important meaning of strategy.

Cytoprotective effect of kidney and liver The University of Michigan reported in 1991 that ACPC protected cultured kidney tubule cells against calcium ionophore-induced lethal cell injury. The protective effects were sustained for long durations (Weinberg *et al*, 1991). Moreover, ACPC protected the isolated perfused rat kidneys against hypoxic injury to the medullary thick ascending limb and slowed functional deterioration in the course of perfusion (Heyman *et al*, 1992). According to their data, this effect is dependent on the dose. ACPC exerted a cytoprotective effect at a concentration of two mmol.

Isolated hepatocytes from rat liver in primary culture rapidly lost viability under hypoxic conditions. Three standard amino acids (glycine, *L*-alanine, and *L*-serine), sarcosine and ACPC significantly decreased hypoxic injury of the hepatocytes at a concentration of 10 mmol/L, but significantly affected neither the ATP content nor the lactate production of the hypoxic hepatocytes (Brecht and de Groot, 1994). The addition of the protective amino acids led to marked membrane alterations (blebs). However, these alterations occurred without loss of viability and were reversible upon reoxygenation after up to four hours of hypoxia (Brecht and de Groot, 1994).

Lipotropism effect Magnesium-pyridoxal-5'-phosphate-glutamate complex (MPPG) has been shown to ameliorate atherosclerotic symptoms induced by hypercholesteremia in rats and especially in rabbits (Schneider, 1987). Soon afterwards, Meyer, Schneider, and Elstner (1992) reported that MPPG interacted with peroxides detectable as increased chemiluminescence and fragmentation of ACPC. Atherosclerosis is attributable to lipid peroxidation and excessive sedimentation in the blood. MPPG might be involved in lipid hydroperoxide metabolism depending on certain amino compounds (ACPC) (Meyer, Schneider, and Elstner, 1992). The anti-lipid role of MPPG was relevant to increase the volume of ACPC. Accordingly speculated, ACPC has the lipotropism effect.

Anti-radiation (mobile phone, microwave, screen etc.) The survival rate and duration of mice radiated by Co^{60} is significantly increased. Fifty healthy mice weighting between 19 and 22 g were randomly divided into five groups (5 male and 5 female mice in each group). The mice were continuously infused with ACPC for two weeks in doses of daily 50 mg/kg. The mice were totally irradiated with ^{60}Co γ rays 15 min in the distance of one meter and a total dose of 0.224 C/kg. As a result, the survival rate of mice doubled (Zhao, 2005).

Protection on brain and nervous system

The data used in the analysis of the neuro-protective effects of ACPC were abstracted from 38 reports, which were performed during 1989 to 2005 in seven different countries – USA, Poland, Spain, England, Israel, Japan, and China. The earliest report is from the USA and the last from Japan.

The neuroprotective actions of ACPC comprise several different points. Among these 38 articles, which demonstrated the positive effects of ACPC on nerves, neurons and the nervous system, 16 of them described the effect of anti-neurotoxicity including NMDA, dynorphin-A, glutamate, and morphine induced neurotoxicity; Five researches indicated the protective effect on cerebral neurological injury induced by ischemia; eight papers published its antidepressant and anxiolytic effects; six articles studied the action of anti-convulsion and -seizures and control of epilepsy; two documents evaluated the activity on facilitating spatial learning and memory; and the last one

determined the effect of reducing alcohol consumption.

Protection on brain neurological impairment induced by ischemia

Based on the five found evidences about the protection on cerebral neuronal cell after ischemia, there are three ischemic injury modes in brain to be protected by ACPC. *In vivo* experiments with mice, the protections of ACPC on acute and chronic brain ischemic injury as well as focal cerebral infarction were tested. All three experiment modes indicated the positive results.

Acute cerebral ischemic injury Decapitated mice were used to observe the effect of ACPC on the gasp duration (from decapitation to stop breath) after acute complete cerebral ischemia. Eighty male mice were randomly separated into following five groups: a normal control group with 0.9% physiological saline (0.1 mL/10 g), a positive control group with Nimodipine (2 mg/kg), and three ACPC groups in low dose (100 mg/kg), middle dose (200 mg/kg), and high dose (400 mg/kg), respectively. Forty minutes after ip administration of each group, the mice were decapitated quickly and their gasp duration and number of times their mouth opened were recorded immediately (Zhao, 2005).

It was found that the gasp duration of mice in the normal control group was between 17 and 21 seconds (Zhao, 2005). Mice in both ACPC (in 100, 200, 400 mg/kg) and Nimodipine groups could prolong gasp duration and increase mouth opening times of decapitated mice. The gasp duration in the normal control group was much shorter than those in ACPC-treated groups ($P < 0.05$) (Table 1). In addition, ACPC groups with middle and high dose showed great significant differences ($P < 0.001$). In comparison with the normal control group, the number of mouth opening times was also significant increased by all ACPC groups, which accounted from about 12 to 14 times in low dose ($P < 0.05$), more than 14 times in middle dose ($P < 0.01$), and much more than 16 times in high dose ($P < 0.001$). The gasp duration of decapitated mice was extended from approximately 18 s to more than 21 s in ACPC low dose group, 24 s in middle dose group and even 26 s in the high dose group (Table 1). Therefore, ACPC can protect the acute brain ischemic injury.

Chronic cerebral ischemic injury Common carotid artery occlusion (CCAO) mice were used to analyze the effect of ACPC on the survival time after acute incomplete cerebral ischemia. Ninety male mice were randomly separated into six groups: a control group (sham operation, common carotid artery only separated but not ligatured) with 0.9% physiological saline (0.1 mL/10 g); a cerebral ischemia model group (bilateral common carotid artery separated and ligatured) with 0.9% physiological saline; a Nimodipine group (2 mg/kg) and three ACPC groups in low dose (100 mg/kg), middle dose (200 mg/kg), and high dose (400 mg/kg), respectively (Zhao, 2005). Thirty minutes after abdominal cavity administration of each group, the mice were anaesthetized with 10% chloral hydrate (0.04 mL/10 g), then the bilateral common carotid artery and the pneumogastric nerve were separated, and both sides were ligatured in the meantime to make acute cerebral ischemia (cerebral ischemia model) (Ye and Li, 1993). The survival time of mice (minute breath less than or equal to five times is death) was recorded and their existence condition within 120 min was observed.

Table 1 Effects of ip ACPC 40 min before ischemia on gasp duration of mice ($\bar{X} \pm s$, $n = 16$)

Groups	Dose / (mg·kg^{-1})	Times of mouth open / n	Gasp duration / s
control	—	12.7 ± 1.6	18.3 ± 1.9
Nimodipine	2	16.9 ± 2.2***	26.4 ± 4.0***
ACPC	100	14.0 ± 1.4*	21.6 ± 2.4*
	200	14.8 ± 1.6**	24.1 ± 4.0***
	400	16.6 ± 2.0***	26.6 ± 3.7***

$^{*}P < 0.05$ $^{**}P < 0.01$ $^{***}P < 0.001$ *vs* control group

It was found that both ACPC (100, 200, and 400 mg/kg) and Nimodipine groups could remarkably prolong the survival time and reduce the mortality within 2 h of CCAO mice. The survival time in the ischemia model group were about six to fifteen minutes (Zhao, 2005), which were much shorter than those in all ACPC-treated groups which had a survival time of more than 30 min (all $P < 0.01$). Furthermore, ACPC groups in middle and high doses have greatly significant difference ($P < 0.001$) compared to the cerebral ischemia model group (Table 2). The 2 h mortality of CCAO mice was decreased from 100% to 93% in the ACPC middle dose group and 80% in the ACPC high dose group. The survival time were expanded from approximately ten minutes to more than one hour and even more than 90 min in the middle and high dose ACPC group respectively. In consequence,

Table 2 Effects of ip ACPC 30 min before ischemia on survival time of mice ($\bar{X}$ ± s, *n* = 15)

Groups	Dose / (mg·kg^{-1})	2 h mortality / %	Survival time / min
control	—	0	120.0 ± 0.0
cerebral ischemia model	—	100	10.0 ± 3.4
ACPC	100	100	29.1 ± 12.0**
	200	93	65.7 ± 27.9***
	400	80	90.6 ± 22.6***
Nimodipine	2	80	93.0 ± 17.7***

** $P < 0.01$ *** $P < 0.001$ *vs* ischemia model group

ACPC can protect the chronic (acute incomplete) brain ischemic impairment.

Focal cerebral infarction To observe the improvement of neurological deficit after focal cerebral infarction of mice by ACPC, the permanent focal ischemic model of middle cerebral artery occlusion (MCAO) was established by inserting nylon thread (nonabsorbable surgical suture, 5/0) through the right common carotid artery into the anterior cerebral artery (Wang *et al*, 2003).

Ninety male mice were randomly separated into six groups: a control group (sham operation, suture was not inserted into brain) with 0.9% physiological saline (0.1 mL/10 g); a focal cerebral ischemia model group (MCAO) with 0.9% physiological saline; a Nimodipine group (2 mg/kg) and three ACPC groups with one low dose (100 mg/kg), second middle dose (200 mg/kg), and third high dose (400 mg/kg) respectively (Zhao, 2005). ACPC and other medicines were all injected through abdominal cavity (ip) within 10 min after focal cerebral ischemia model established.

Within 24 h after operation, nervous function deficiency was observed and recorded the number of survival mice of each group recorded to calculate the survival rate. Neurological deficit evaluations were carried out after 6 and 24 h according to MCAO using a 5-point behavioral rating scale (Longa *et al*, 1989; Bederson *et al*, 1986). Grade 1 to 4 is an effective animal model. The higher the grade is, the more obvious neurologic deficit symptoms are (Table 3).

Twenty-four hours after MCAO, the brains of survival mice were removed to determine the cerebral index and infarcted area. The moist brains were weighed and determined cerebral index with the formula: cerebral index = weight of moist brain × 100 / body weight.

Table 3 Neurologic examination grading system

Level of neurologic deficit	Grade	Behavioral expression
normal	0	no observable deficit
moderate	1	incomplete extension of injured forelimb or forelimb flexion
severe	2	side lurch, decreased resistance to lateral push (and forelimb flexion) without circling
	3	same behavior as grade 2, with circling
	4	nnwalkable, disorder of consciousness

The infarction size was measured by triphenyltetrazolium chloride (TTC) stain and image analyzer. TTC produces a red product upon reaction with the respiratory enzymes (dehydrogenases) present in non-infarcted tissues. Irreversibly damaged tissues, lacking dehydrogenases, do not form red reaction products (Lundy *et al*, 1986). Six 1 mm thick coronal slices were cut and immediately placed in the TTC solution (20 g/L), incubated for 30 min with 37 ℃ constant temperature protecting from light (Zhao, 2005). The TTC was found to react selectively only with non-infarcted cerebral tissue. Normal cerebral tissue appeared rose color and the infracted area appeared a white color. The brain slices stained with TTC were additionally put into the 10% methanol solution for more than two hours before they were set on the slides to be measured and determined the infarct volumes through computer-assisted imaging analysis (Liang *et al*, 2003).

The outcome of the survival rate of MCAO mice was in the focal cerebral ischemia model group 92%, compared to which was 100% in the control group and all other medicine groups (Zhao, 2005).

Twenty hours after MCAO, the neurological deficit score was more serious in the focal cerebral ischemia model group with grade 3 compared to grade 2.6 6 h after MCAO. However, the grades improved in all three ACPC groups with grade 2.2, 1.9, 1.9, respectively and the Nimodipine group with grade 2.3. Furthermore, each ACPC group could significantly improve the symptoms of mice neurological deficit both in 6 and 24 h comparing to focal cerebral ischemia model group, especially 24 h after MCAO ($P < 0.01$, 0.001) (Table 4).

There was obvious cerebral tissue edema in the focal cerebral ischemia model group, which manifested a distinct increase of cerebral index with about 1.32,

Table 4 Effect of ACPC on neurological deficits after focal cerebral ischemia in mice ($\bar{X} \pm s$)

Groups	Dose / (mg·kg^{-1})	*n*	Neurological deficit grade	
			6 h	24 h
control	—	15	0.0 ± 0.0	0.0 ± 0.0
cerebral ischemia model	—	11	2.6 ± 0.7	3.0 ± 0.6
ACPC	100	11	2.5 ± 0.7	2.2 ± 0.8**
	200	13	2.4 ± 0.5	1.9 ± 0.8***
	400	12	2.6 ± 0.7	1.9 ± 0.7***
Nimodipine	2	12	2.6 ± 0.5	2.3 ± 0.6*

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$ *vs* ischemia model group

compared with control group (about 1.16) in great significant difference ($P < 0.001$) (Table 5). By all ACPC groups (1.22, 1.21, 1.18) and the Nimodipine group (1.21), the cerebral index went down significantly ($P < 0.01$) compared to the focal cerebral ischemia model group. The higher the cerebral index is, the more severe the degree of cerebral tissue edema implies. It indicated that ACPC could be resistant to cerebral tissue edema appearance caused by cerebral ischemia.

Table 5 Effect of ACPC on cerebral index 24 h after focal cerebral ischemia in mice ($\bar{X} \pm s$)

Groups	Dose / (mg·kg^{-1})	*n*	Cerebral index / (×10^2 g·g^{-1})
control	—	15	1.1653 ± 0.0816
cerebral ischemia model	—	11	1.3250 ± 0.1134△△△
ACPC	100	11	1.2167 ± 0.0610**
	200	13	1.2072 ± 0.0727***
	400	12	1.1801 ± 0.0576***
Nimodipine	2	12	1.2114 ± 0.0725***

△△△ $P < 0.001$ *vs* control group; ** $P < 0.01$ *** $P < 0.001$ *vs* ischemia model group

Only the brain slices of MCAO mice stained with TTC could be seen two well-defined areas of red and white. The red region showed normal brain tissues and the white region was infarction focus. It was found that in the control group the brain slices were all red without white infarct focus, whereas there were extensive white infarct areas in the focal cerebral ischemia model group. But the size of infarct focus in each ACPC group and Nimodipine group was greatly reduced (Fig. 4). The cerebral infarct volume was about 132.45 cubic millimeters in the ischemia model group, which was much smaller in the Nimodipine group with 90.83 mm^3 and all ACPC groups with 100.60 mm^3 in low dose, 87.38 mm^3 in middle dose and 84.08 mm^3 in high dose (Table 6). Consequently, the corresponding cerebral infarct rate was significantly decreased by the three ACPC groups with 25% ($P < 0.05$), 21.43% ($P < 0.001$), and 19.12% ($P < 0.001$), respectively compared to focal cerebral ischemia model group with 32.37% (Table 6). Both infarct volume and infarct rate showed great significant difference ($P < 0.05$, 0.01, 0.001) by ACPC groups in comparison with the focal cerebral ischemia model group. It indicated that ACPC could protect brain tissues from the impairment of cerebral ischemia and improve the state after injuries.

On the whole, the infarct volume, cerebral index,

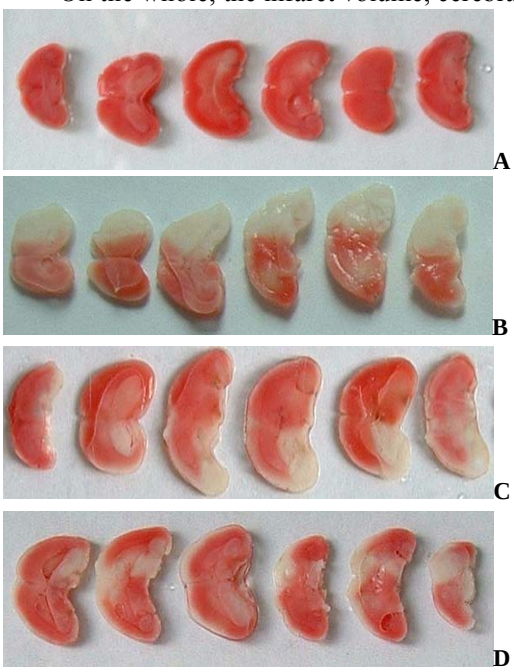


Fig. 4 Coronal brain slices of mice (TTC stain)

A: control group; B: ischemia model group; C: ACPC high dose group (400 mg·kg^{-1}); D-Nimodipine group

It is obvious that in the image C and D the white infarct volume are reduced and red normal brain tissues areas are increased comparing with image B (almost all white). Therefore, ACPC can protect brain tissues from the injury of cerebral ischemia and also improve symptoms of neurological deficit

Table 6 Effect of ACPC on infarct volume 24 h after focal cerebral ischemia in mice ($\bar{X} \pm s$)

Group	Dose / (mg·kg^{-1})	*n*	Infarct volume / mm^3	Infarct rate / %
control	—	15	0.00±0.00	0.00±0.00
cerebral ischemia model	—	11	132.45±49.32	32.37±8.70
ACPC	100	11	100.60±40.11*	25.00±5.18*
	200	13	87.38±34.54**	21.43±7.29***
	400	12	84.08±27.16**	19.12±6.52***
Nimodipine	2	12	90.83±27.41**	22.79±8.26**

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$ *vs* ischemia model group

mortality and neurological deficit score in the focal cerebral ischemia model group were higher than those in ACPC-treated groups ($P < 0.05$). ACPC could significantly improve the symptoms of mice neurological deficit through reducing infarct volume and decrease the cerebral index.

Anti-neurotoxicity both *in vivo* and *in vitro* Previous studies showed the protective effects on brain against neurons death after ischemia *in vivo*. Such results could also be correlated with *in vitro* assays in which ACPC protected neuronal cells against several substances induced neurotoxicity. The effect of ACPC on anti-neurotoxicity was summed up with mainly four kinds of neurotoxicity according to the 16 selected literatures, which are anti-NMDA, -dynorphin-A, -glutamate, and -morphine induced neurotoxicity.

Anti-NMDA-induced neurotoxicity ACPC, a glycine partial agonist, is an effective antagonist at NMDAR. ACPC antagonizes a variety of NMDAR mediated actions *in vivo* and *in vitro*, presumably under conditions in which synaptic concentration of glycine is at or near saturation (Lin *et al*, 1998). Sustained exposure to ACPC alters NMDAR function and subunit composition (Fossom *et al*, 1995a; Choi, 1988). They first demonstrated that sustained (24 h) exposure to a glycinergic ligand can alter the expression of RNAs encoding NMDAR subunits. These results may have important implications for the treatment of neurodegenerative disorders. Afterwards, it was similarly reported that chronic administration of ACPC could alter the expression of NMDAR subunit mRNAs (Bovetto *et al*, 1997). In a parallel manner, sustained (20 h) exposure to ACPC has been shown to alter NMDAR agonist and antagonist potencies and significantly reduce NMDA-induced neurotoxicity in cultured spinal cord neurons (Lin *et al*, 1998). Such alterations in NMDAR subunit composition might explain the neuroprotective effects produced by chronic ACPC (Bovetto *et al*, 1997).

The effect of systemic treatment with ACPC on convulsions and neurodegeneration induced by intrahippocampal (ihp) injection of NMDA was investigated in mice by Zapata in 1996. After five days of ip NMDA infusion, 80% to 100% of the pyramidal cell was dead in the CA1 region of the hippocampus. By a pretreatment with ACPC, the lethal effects of NMDA were prevented and significantly reduced seizure induction. ACPC reduced cell death to 40% of that induced by a dose of NMDA in 6 nmol that damaged 80% of hippocampal CA1 neurons in untreated animals. These findings provided further evidence that ACPC can reduce NMDAR function *in vivo* and suggested that it may be useful anticonvulsant and neuroprotective agents (Zapata *et al*, 1996). The experiment results were consistent with previous researches, for instance, as early as 1989, Skolnick pointed out that ACPC blocked (ED_{50} 234 mg/kg) the convulsions and deaths produced by NMDA (125 mg/kg) in a dose dependent fashion and suggested that ACPC may be useful in the treatment of neuropathologies associated with excessive activation of NMDAR coupled cation channels (Skolnick *et al*, 1989).

Anti-dynorphin-A-induced neurotoxicity Several evidences of studies supported that lumbar subarachnoid injection of dynorphin-A causes an ischemia-induced neuronal degeneration and persistent hindlimb paralysis (Faden and Jacobs, 1984; Herman and Goldstein, 1985, Long *et al*, 1988; Stevens and Yaksh, 1986). Dynorphin-A has an antinociceptive action at the level of the spinal cord (Herman and Goldstein, 1985). Activation of the NMDAR complex is essential for dynorphin A-induced spinal cord injury (Long *et al*, 1994). Thirty minutes pretreatment of rats with ACPC (100 and 200 mg/kg, ip) prior to dynorphin A significantly eliminated the persistent hindlimb motor deficits and neuropathological changes produced by 20 nmol of this peptide. When given instead as six daily injections (200 mg/kg, ip) followed by an injection-free day, ACPC also significantly improved neurological recovery following dynorphin-A injection (Long and Skolnick, 1994). These results were supported earlier and indicate that first, activation of the NMDAR complex plays a critical role in mediating dynorphin A-induced rat spinal cord injury; Second, ACPC provides an effective means of antagonizing excitotoxic phenomena; And third, chronic administration of ACPC can elicit a persistent change in the NMDAR complex. Thus, glycine partial agonists associated with the NMDAR complex such as ACPC have been demonstrated to block or reduce NMDAR-mediated actions both *in vivo* and *in vitro* (Boje *et al*, 1993;

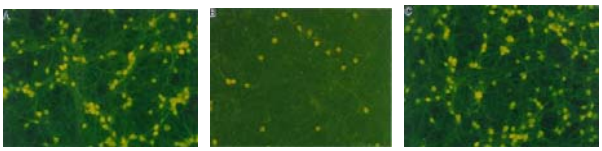
Trullas and Skolnick, 1990; Priestley *et al*, 1990). In particular, the neuroprotective actions of ACPC in both primary neuronal cell cultures (Boje *et al*, 1993) and global cerebral ischemia (Von *et al*, 1992) prompted an examination of this compound on recovery from dynorphin A-induced spinal cord injury.

Anti-glutamate-induced neurotoxicity

Glutamate is not only the principle excitatory neurotransmitter in the brain but also a potent endogenous toxin, which lead to the neuronal injury and even death in a variety of pathologic conditions. ACPC has the ability to attenuate glutamate-induced neurotoxicity under both *in vivo* and *in vitro* conditions (Layer, Bland, and Skolnick, 1993). Particularly in primary granule cell culture, ACPC significantly attenuates neurotoxicity induced by low to moderate glutamate concentrations (Boje *et al*, 1993). Consistent with this finding, Fossom *et al* (1995) also indicated that ACPC significantly reduced glutamate-induced neurotoxicity in cerebellar granule cell cultures. ACPC was most effective in blocking neurotoxicity at glutamate concentrations producing low to moderate levels of cell death.

In vitro experiment, ACPC could reduce the excitotoxicity of glutamate and protect against neuronal apoptosis induced by glutamate. So that significantly increased the survival rate of cerebella granular neurons (Fig. 5); vanished the phenomenon away from pycnosis, aggregation and fragmentation of cell nucleus (Fig. 6) and that agarose gel electrophoresis pattern of neuronal DNA fragmentation presented "ladderlike" diagram (Fig. 7) induced by 200 μmol/L glutamate (Zhao, 2005).

Survival rate of cerebella granular neurons The in culture for eight days cerebella granular neurons were administrated with 200 μmol/L glutamate and observed with phase contrast microscope after 12 h. It was found to be an obvious shrinkage of cytons and karyopyknosis. The neurons were then observed with a fluorescence microscope and taken pictures of and counted. With an increase of the concentration of ACPC, the survival rate of neurons was improving constantly until the rate was close to rate of the control group (Zhao, 2005). It could be seen on these pictures (Fig. 5) that there were much less survival neurons in the picture B (glutamate) than in A (control group); however, because of the treatment with ACPC (Fig. 6C), more than 97% (Table 7) neurons were protected against cell death. The contrast was remarkable.



A B C

Fig. 5 Effect of ACPC on the apoptotic action of glutamate against neurons

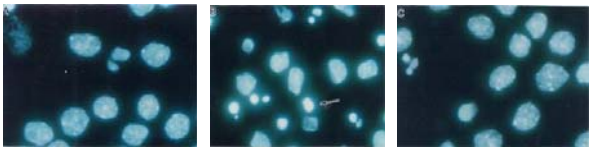
A: control group; B: 200 μmol·L^{-1} glutamate; C: 1 mmol·L^{-1} ACPC + 200 μmol·L^{-1} glutamate

Table 7 Effect of ACPC on the apoptotic action of glutamate against neurons ($\bar{X} \pm s$, n = 3)

Group	Dose / mmol·L^{-1}	Survival rate / %
control	—	100.0 ± 0.0
glutamate	0.2	17.2 ± 1.5$^{\triangle\triangle}$
ACPC + glutamate	0.1 + 0.2	20.6 ± 1.1*
	0.5 + 0.2	62.0 ± 1.6**
	1.0 + 0.2	97.4 ± 1.0**
Nimodipine	10	98.2 ± 1.2**

$^{\triangle\triangle}$ $P < 0.001$ *vs* control group; * $P < 0.05$ ** $P < 0.001$ *vs* glutamate group

Effect on karyomorphism of neurons Hoechst 33258 fluorescent staining (Fig. 6) displayed that 200 μmol/L glutamate made remarkably pycnosis, aggregation and fragmentation of cerebella granular neuronal nucleus (Fig. 6B), comparing with the additional administration of 1 mmol/L ACPC, which returned the cell nuclear of neurons almost to normal and looked like the same as the control group (Fig. 6C).



A B C

Fig. 6 Nuclear analysis of apoptotic neurons with Hoechst 33258

A: control group; B; 200 μmol·L^{-1} glutamate; C: 1 mmol·L^{-1} ACPC + 200 μmol·L^{-1} glutamate

Agarose gel electrophoresis of DNA fragmentation analysis

DNA of neurons was cut into different size fragments in a given locus. The agarose gel electrophoresis pattern of neuronal DNA fragmentation (Fig. 7) presented a "ladderlike" diagram, which is the most important mark of cell apoptosis (Zhao, 2005). The results indicated that cell DNA in the control group on agarose gel electrophoresis showed a band of great molecular weight, which explained that their cell

apoptosis was not to happen. In contrast, in the 200 μmol/L glutamate group, cell DNA electrophoresis showed a clear DNA ladder. Nevertheless, in the 200 μmol/L glutamate + 1 mmol/L ACPC group, the cell DNA was the same as in the control group.

Fig. 7 Agarose gel electrophoresis of DNA fragmentation analysis

M: Maker; 1: control group; 2: 200 μmol·L^{-1} glutamate; 3: 1 mmol·L^{-1} ACPC + 200 μmol·L^{-1} glutamate; 4: 10 μmol·L^{-1} Nimodipine

Anti-morphine-induced neurotoxicity ACPC acts as a protection aginst toxicity induced by morphine. Kolesnikov (Kolesnikov *et al*, 1994) reported that ACPC prevented tolerance to the mu opioid morphine and the delta ligand [D-Pen2, D-Pen5] enkephalin (DPDPE) when co-administered with the opioid. But the actions of ACPC are restricted to tolerance. When given alone, ACPC had no analgesic actions in the tailflick assay, and it did not change morphine's ED_{50} in naive mice. They also found that ACPC could reverse preexisting tolerance. For instance, when mice were made tolerant to morphine over five days and then received ACPC along with their morphine, analgesia returned to naive levels within three days despite the continued administration of morphine. The actions of ACPC on opioid tolerance, they thought, correspond closely with those previously described with both competitive and non-competitive NMDA antagonists.

Antidepressant and anxiolytic effects

Przegaliński *et al* (1997) examined the antidepressant-like activity of ACPC after its ip and intrahippocampal administration by using the forced swimming test (Porsolt test) in rats. ACPC (200–400 mg/kg), administered ip, produced a dose- dependent and significant reduction of the immobility time in the forced swimming test. A similar effect was also observed after ihp administration of ACPC (10 and 30 μg). The results indicated that ACPC exhibited an antidepressant-like activity like imipramine (used as a reference drug). Through the forced swimming test with rats; moreover, it seemed to show that the hippocampus may be one of the neuroanatomical sites involved in this effect (Przegaliński *et al*, 1997).

Using the conflict drinking test (Vogel test) as a model in rats, the anxiolytic-like activity of ACPC was also evaluated after the administration of both ip and ihp (Przegaliński *et al*, 1996). ACPC exhibited an anxiolytic-like activity after both ip (100–200 mg/kg) and ihp (3–30 μg) administration. These results, as well as the literature data on the lack of motor-impairing effects of ACPC, indicated that ACPC seemed to be more advantageous than other competitive NMDAR antagonists as a potential therapeutic agent in the treatment of anxiety disorders. Furthermore, they also showed that the hippocampus may be one of the neuroanatomical sites of the anxiolytic-like effect of ACPC (Przegaliński *et al*, 1996).

The anxiolytic and antidepressant activity of ACPC was also studied both in acute and chronic or repeated administration. In the conflict drinking test, acute administration (24 h or 4 d later) of ACPC (200 mg/kg) increased fivefold compared to the effect of diazepam (as anxiolytic reference drug). On the other hand, an only about threefold increase was observed in rats treated repeatedly with the same dose of the drug (200 mg/kg daily; 14 d) (Przegaliński *et al*, 1999). A single injection of ACPC (400 mg/kg) reduced by 40% less than imipramine the immobility time in the forced swim test, while in rats treated repeatedly with ACPC (400 mg/kg daily; 14 d) and challenged with the same dose 24 h or 4 d later, the drug either produced no significant effects or reduced the immobility time by 50%, respectively (Przegaliński *et al*, 1999). This indicated that tolerance developed to the anxiolytic- and, particularly, to the antidepressant-like activity of ACPC in rats after repeated or chronic (14 d) treatment. It was explained that chronic administration of ACPC produced a behavioral tolerance putatively through an adaptation of the NMDAR complex (Nowak *et al*, 2000; Lopes, Neubauer, and Boje, 1997; Skolnick *et al*, 1992).

However, in a chronic mild stress model of depression, a substantial decrease in consumption of a palatable sucrose solution was observed over time in rats subjected to a variety of mild stressors (Papp and Moryl, 1996). Chronic (five weeks) ACPC treatment gradually reversed chronic mild stress-induced deficit

in sucrose intake. This decrement can be commonly reversed by chronic administration of antidepressant drugs such as imipramine. The effect's magnitude of ACPC was comparable to that observed following similar administration of imipramine (10 mg/kg). ACPC exhibited a dose dependence in the time duration for reversion. Consequently, the statistically significant effect of the low dose of ACPC (100 mg/kg) was first observed after four weeks of treatment (comparable to the 3–5 weeks required for imipramine), while only two weeks of treatment was required in the group receiving a higher dose (200 mg/kg) of ACPC. And the reverse, like imipramine, persisted for at least one week following cessation of treatment (Papp and Moryl, 1996). This finding was consistent with the observation that ACPC reduced immobility in the forced swim test to the same extent as imipramine (Trullas and Skolnick, 1990). ACPC appeared to be as efficacious as imipramine and produced a full and rapid resolution of stress-induced reductions in the chronic mild stress model. Thus, Papp and Moryl (1996) suggested that ACPC may have antidepressant properties comparable to conventional drugs, but with a faster onset of action. Moreover, Phase Ⅰ clinical trials indicated that functional NMDAR antagonists such as eliprodil and ACPC were devoid of psychotomimetic-like actions, (Cherkofsky, 1995; Patat *et al*, 1994).

The zwitterionic character of ACPC could limit both penetration into the central nervous system and oral availability (Trullas *et al*, 1991). They determined that parenterally and orally administered ACPC were equipotent in reducing immobility in the forced swim test, an action manifested for at least 6 h; comparatively, in the anxiolytic test, ACPC was active for one to two hours after parenteral administration. These findings suggested that ACPC may constitute a novel class of antidepressant or anxiolytic agents.

Facilitating spatial learning and memory

While ACPC was reported active in animal models used to evaluate potential antidepressants and anxiolytics, effects on learning and memory of this compound were also researched during the same period. The activation of NMDAR has been hypothesized to mediate certain forms of learning and memory functions (Viu *et al*, 2000). Popik and Rygielaka (1999) investigated the effects of ACPC on spatial learning in the Morris water maze. On a schedule of 12 learning trials, one trial per day, mature male Wistar rats (3 months of age) rapidly acquired the task. Electroconvulsive shocks applied after each of the learning trials markedly inhibited the consolidation of spatial memory. The administration of ACPC (250 or 400 mg/kg) 20 min before each of the learning trials did not affect the acquisition of spatial learning. In contrast, aged (16 months old) male Wistar rats demonstrated difficulties in the acquisition of spatial learning task. In these cases, ACPC administered 20 min before each of the learning trials a dose of 400 instead of 250 mg/kg, this facilitated the acquisition of spatial memory. But ACPC did not affect the strength of spatial memory as assessed at the end of conditioning, by measuring swimming behaviour of rats in the pool with the platform removed. It was suggested that ACPC may only alleviate learning deficits observed in the elderly (Popik and Rygielaka, 1999).

As a NMDAR partial agonist, pretreatment with ACPC prevented memory deficits induced by hypoxia (exposure to 7% oxygen) and the convulsant drug pentylenetetrazole (PTZ) (45 mg/kg), but did not affect the sedative scopolamine-induced learning impairment (Viu *et al*, 2000). In addition, ACPC prevented consolidation deficits evoked by a nonexcitotoxic concentration of a competitive inhibitor of glutamate transport that increases extracellular levels of glutamate. Furthermore, ACPC facilitated both acquisition (learning) and consolidation (memory) of inhibitory avoidance training, an effect that was dose-dependent and reversed by glycine. These results indicated that memory deficits induced by both hypoxia and PTZ involved NMDAR activation. Moreover, it demonstrated that ACPC prevented memory deficits of inhibitory avoidance learning by affecting consolidation, but not acquisition processes (Viu *et al*, 2000).

Anti-convulsion and-seizures and control of the epilepsy

The nucleus reticularis pontis oralis (RPO) is necessary for the expression of tonic hindlimb extension (THE) in maximal electroshock (MES) seizures of rats. Bilateral RPO microinfusion of the competitive NMDA antagonist ACPC inhibited the response to MES, but it did not significantly affect the THE response (Peterson, 1995). The results of his study supported a hypothesis that the RPO is a site of

anticonvulsant drug action in MES and indicated that NMDA antagonist (ACPC) action regulates the anticonvulsant activity mediated by the RPO.

ACPC has additional neuroprotective effects in lithium-pilocarpine status epilepticus (Peterson, Purvis, and Griffith, 2004). The status epilepticus (SE) could be induced in rats by lithium-pilocarpine (Li-pilo). ACPC administered five minutes after the SE onset produced significant neuroprotection in cortical regions, amygdala and CA1 of the hippocampus (Peterson, Purvis, and Griffith, 2004).

In the forced swim test (FST), ACPC produced anti-depressant-like actions. However, seven daily injections of ACPC (200–400 mg/kg) abolished this effect (Skolnick *et al*, 1992). The loss in effectiveness of ACPC required seven days of treatment to become fully manifest, and was reversed by discontinuing treatment. The chronic treatment with ACPC did not affect its actions in FST, but it significantly attenuated the convulsant and lethal effects of NMDA (125 mg/kg). It was suggested that repeated administration of ACPC may effect an "uncoupling" of NMDA and glycine receptors, resulting in an apparent desensitization of the behavioral actions of substances acting at these sites (Skolnick *et al*, 1992). In short, ACPC has the effect of anticonvulsant and antiepilepsy.

Reduction of alcohol consumption

The function on reducing alcohol consumption of ACPC is a plus for its protective effects to the nervous system. Both systemically and single dose administered ACPC significantly reduced ethanol consumption in a dose-dependent manner. Indirect measures of general appetitive behavior showed no effect of ACPC on weight or water intake, which suggested that this effect of ACPC may be specific to ethanol (Stromberg *et al*, 1999). They further indicated that the effect of ACPC on ethanol consumption was in part due to its interaction with NMDAR located in the nucleus accumbens. However, the precise mechanism of ACPC's effect on ethanol drinking was unclear. One possibility was that ACPC alters the neuronal hyperexcitability of NMDAR that occurs after chronic ethanol use (Grant *et al*, 1990). The finding of Stromberg (Stromberg *et al*, 1999) was the initial evidence demonstrating that ACPC could reduce consumption of ethanol in rats. It was also suggested that since ACPC has been shown to have neuroprotective effects and did not show the psychotomimetic properties of other drugs observed with NMDAR agents. Thus, ACPC might be helpful in future clinical studies designed to reduce alcohol use and significant in the treatment of recovering alcoholics. As widely known, alcohol abuse produces serious psychotomimetic side effects and neuronal hyperexcitability, which leads to a temporary state of mental confusion and clouded consciousness, for example hallucinations, delusions, trembling, incoherent speech and so on.

Cardiovascular system effects

Recent studies have demonstrated that several antagonists of NMDAR have important cardiovascular effects (Muir and Lees, 1995), such as regulation of mean arterial pressure and splanchnic sympathetic nerve activity (Lin, Tsao, and Wang, 1995; Mills *et al*, 1990). Besides all the above descriptive and already definite effects and functions of ACPC, it was recently discovered that ACPC has important actions on cardiovascular system involved control of hypertension, antioxidant and vascular relaxation effects (Gao *et al*, 2007) as well as prevention and treatment of thrombosis and stroke.

A current study indicated that ACPC caused hypotensive and antioxidant effects in stroke-prone spontaneously hypertensive rats (SHRSP) (Gao *et al*, 2007). Systolic blood pressure (SBP) (Fig. 8) and mortality of stroke (Fig. 9) were significantly lower in the ACPC group than in the control group (peritoneal injected with distilled water). Urinary Na^+ and Cl^- excretion and plasma superoxide dismutase (SOD) activity were increased in the ACPC group. The administration of ACPC also drastically increased the expression of heme oxygenase-1 (HO-1) in the hippocampus and cerebral cortex, which exerts antioxidant and vascular relaxation effects, increases cerebral blood flow and suppresses hypertension. These results provided an evidence of the normalization by ACPC of blood pressure elevation and mortality of stroke through antioxidant effects in SHRSP. Furthermore, the increase of HO-1 expression induced by ACPC not only enhanced carbonic oxide (CO)-dependent relaxation of the cerebral arteries and improved the regulation of blood pressure, but also generated bilirubin, which exerts antioxidant cytoprotection (Gao *et al*, 2007).

The China National Patent of Invention "Application of ACPC in preparation of medicine against cardiovascular

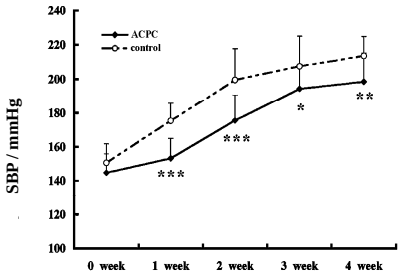


Fig. 8 SBP of SHRSP that were injected with distilled water (control group) or ACPC by ip (ACPC group) at 50 mg·kg^{-1}·d^{-1} for 4 weeks

*P < 0.05 **P < 0.01 ***P < 0.005 *vs* control group

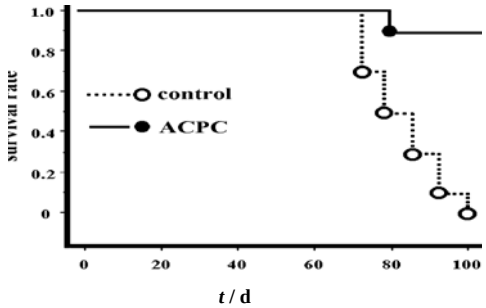


Fig. 9 100-d survival rate after administration of ACPC

diseases" provided a new kind of therapeutic action of ACPC (Zhu, 2007). This invention revealed that ACPC has some new pharmacological effects on anti-high blood pressure, anti-thrombosis and prevention and treatment of stroke. It is of great significance to hypertension, cardio-cerebral thrombosis and stroke, effective protection to the heart, kidneys and brain with non-toxic side effects.

The SBP decreased by 20 mmHg in the ACPC group compared to the control group, and the diastolic blood pressure (DBP) was lower by 10 mmHg in the ACPC group. The cerebral blood flow (CBF) determination was measured in the ACPC group with 25% (P < 0.05) volume increase in contrast to the control group in the ratio of 1.5 to 1.2. One hundred days into the experiment, the incidence of stroke was 100% (all ten rats) in the control group and only 20% (two rats) in the ACPC group. When comparing with incidences, the mortality of stroke was significant lower in the ACPC group, in which only one rat (10% mortality) died on the day 80 and the other nine rats were still alive after 100 d; However, all ten rats (100% mortality) in the control group were dead in 80 d. Moreover, ACPC effectively protected the heart against hypertrophy and the kidneys from nephrosclerosis and nephrarctia. During the fourth week of ACPC administration, 24 h urinary nitrogen oxide (NO) and other excretions were determined to significantly decrease by about 50% (P < 0.05) with 0.76 μmol in the ACPC group and 1.36 μmol in the control group.

Discussion

Although the bioactivities and pharmaceutical effects of ACPC were world widely reported by a large number of publications from various countries since the fifties of last century, there is no current literature of comprehensive systematic review or summary about ACPC available, which would introduce and sum up its excellent activities and functions. For the purpose of this systematic review, a total of 279 international studies were selected and identified, 38 articles of them concerning neuroprotective effects of ACPC were focally analyzed with precision.

ACPC has lots of bioactivities and medical physiological actions in many different respects. On the whole, it could be divided into two large parts, one part is the effects on plants and the other part is that on animals and humans.

ACPC is a natural non-protein amino acid which originally occurs in plants, above all, in tomatoes, kiwis, and celery. Regarding the influences of ACPC on plants, in the first place, ACPC as an endogenous plant growth regulator (Abeles, 1973), regulates many aspects of plant development and ripeness, such as germination, blooming, fruiting, fruit sweetening, senescence, chlorophyll content, and sex ratio of floral organs of plants, etc. In the next place, ACPC can protect the plant against being encroached by virus or bacteria, ie, protect agriculture from disaster, which greatly benefits to agriculture.

Research reports from various countries indicated that ACPC widespread exists in higher plants. Cameron *et al* (1979) once gave exogenous administration of ACPC to the tissue slices of roots, stems, leaves, flowers, and fruit of celery, tomatoes and 16 other kinds of vegetables. The results showed a significant increase of ethylene content, which was more than 1000 times as much as the blank experiment (control) within the plant tissues. It was indicated that exogenous ACPC strongly promoted the biosynthesis of endogenous ACPC and its metabolite ethylene, ie, exogenous ACPC in low content could produce vegetables or fruits of rich endogenous ACPC.

Schroeder *et al* (1980) made a series of comparison to ACPC with its derivatives ACPC ester, salt, hydrochloride, hydrochlorate ester, *N*-formyl-ACPC, etc., and found that the majority of them had the same biological activity on the growth inhibition to zymolyte of soybean, barley and wheat.

In addition, Zhu *et al* (1998) reported that ACPC remarkably enhanced the sprouting power and germination percentage of the seeds; improved the quality of the seedlings; promoted the root sprouting, the photosynthesis and the ability of drought-resistance; And fastened the speed of grain-filling, so as to increase the percentage of earbearing tiller, seed set propagation coefficient and the thousand grain weight of rice. The rice production increase of ACPC was 8% to 15%, which surpassed most of the existing plant growth regulators.

However, due to the limited length of this thesis, not every effect of ACPC on plants can be discussed in detail. Moreover, this aspect is not the central topic of this work. Therefore, we carefully chose the two representative effects or examples of disinfection and blooming regulation to describe the massive effects of ACPC on plants. The anti-virus and -bacteria function is effective for many crops and fruits besides paddy rice, such as wheat, tea, apple, kiwifruit, and tomato as well. Because ACPC has disinfection and desinsection effects, it plays a very important role so that it could be used instead of common agriculture chemical. It is well known that the problem of pesticide residue on grain, vegetables or fruit still remains. The currently available agrochemical still has residue, toxic side effects and are even poisonous. In contrast, ACPC is free from pollution and has no residues or toxic side effects. Thus, it is of great significance to the pesticide residue problem of agricultural produce and human food security that ACPC should be developed as a new agent of plant protection.

The second example for regulating action of ACPC on plants is the blooming regulation, which might supply the demand to grasp the season and quantity of flower opening. The process of earlier or later flower opening is an import link to plant development and fructification with regard to practical utility. On the other side, from the perspective of sight enjoyment, being sure of the flowering stage, for instance, is essential to meet the time step selection of certain exhibitions. Furthermore, anthesis and quantity of flowers influence the output of fruit. This effect of ACPC would be of great value to scientific research on plants.

In summary, ACPC might be of three great meanings to the world: protecting agriculture from disastrousness with nuisanceless, raising the agricultural yield and benefit to public health by agricultural product of rich endogenous ACPC.

In the aspect of effects to animals and humans, ACPC acts in following main fields of bioactivities and pharmaceutical effects: Firstly, promoting the animal growth and improving reproductive quality, precisely fowl or poultry, silkworm and quality of eggs; Secondly, protection on brain and the nervous system, for instance the neuroprotection against brain injury, that are caused by ischemia or hypoxia; Thirdly, protective effects on cerebro- and cardiovascular system, for example the control of the hypertension, prevention and treatment of thromboses or heart attacks; Fourthly, protection of internal organs and their cells from impairment under hypoxic condition, such as kidney and liver; Fifthly, anti-infection activity, namely anti-virus, bacteria, and parasitic diseases specially *E. coli*, *S. aureus*, and *Candida albicans* as well as several viruses such as NDV-LaSota (like H5N1), Newcastle Disease Virus; finally, the anti-radiation function of mobile phone, microwave, screen and television, etc.

Critical appraisal of previous investigations

Lack of clinical trials ACPC has shown a variety of bioactivities and pharmaceutical effects. However, results of this literature review indicated that there are many limitations in terms of evidence-based efficacy of ACPC in humans. First of all, previous researches were mostly small, experimental studies with animal models or cell cultures. Most animal models used mouse, rat and rabbit. Only Cherkofsky (1995) made his study also with humans, namely average male volunteers. There were no large clinical trials or database to report the effects of ACPC in humans. It is not enough to demonstrate the efficacy of ACPC on the human population level.

Clinical trial is a rigorously controlled test of a new drug or a new invasive medical device on human subjects. On account of difference in experimental subject, the gap between animal experiment and clinical trial is great in metabolism, reaction, efficacy etc. of the

test substance. For instance, some cosmetic products or ingredient should be tested in the human body rather than in animals. To study the effect of a new drug on human body, medical experiments in humans are essential to be carried out. These human experiments are an important part of medical research. Many volunteers would participate in the clinical trials of medical treatment. Some people are voluntary to become the testee of preclinical medicine and biological experiments.

The effectiveness in animal experiments can not ensure in clinical trials still a positive result. Although ACPC as a neuroprotective agent has entered into clinical trials in the USA since 1995, it still remains in the phase one now. Currently existing neuroprotective agents have great variety and hundreds of drugs were found. However almost all of the neuroprotective agents were effective in animal experiments, but they worked badly or nothing in clinical trials (Degraba and Pettigrew, 2000). Moreover, some drugs because of their severe side effects were limited in the clinical application (Rogawski, 2000). The treatment of neuroprotection showed a serious discrepancy between clinic and theory. Therefore, in spite of the positive effects of ACPC on animals, its influences in humans are still not determinate.

On the other side, clinical trials require a large amount of human, financial and material resources. Lots of money and time would be invested. ACPC as the trial substance should be adequately supplied. Nevertheless, on this point, there are two primary difficulties of ACPC, precisely the dosage problem and financial problem.

The demanded administration dose of ACPC is too much like a drug. Usually 200–400 mg/kg should be used for efficacy. An average adult in weight of 60 kg would need to take 12–24 g ACPC. This is unusual and inconvenient because of the high dose to do some large clinical trials with people and also be developed into a new drug. As a medicine, the volume would be oversized and unpractical.

In addition, the preparation of ACPC is very difficult, both in extraction from plants of rich endogenous ACPC and in chemical synthesis. The problem is high cost but with low yield. Mass production of ACPC is not easy to get, which is also an economic problem for the research. This is another study limitation of the application of ACPC on human body.

Incomplete study on activities of ACPC

Researches on activities and functions of ACPC have not reached maturity. Except the neuroprotective effect, actions in other aspects were not reported by plenty of articles. The results of this present thesis indicated that only the effects on plants and neuroprotection of animals were researched to a certain extent. The other functions of ACPC, especially in the aspect of medical activities, were barely reported by a few studies.

These effects such as anti-radiation, anti-virus, and bacteria and protection of kidney and liver as well as controlling or prevention of hypertension and stroke are all greatly significant to medical research and public health. However, the exploration of these researches is still in its infancy. So far, only some scientists discovered these other activities of ACPC compared to neuroprotection since ACPC has been studied for about 50 years. It could not be definite if they were just hit upon in the experiment or ACPC really has many excellent activities. Thus, effects of ACPC in these aspects could not be determinate without a quantity of reports. Whether ACPC has other more actions, which are not known so far, remains still in question. In consequence, study on each activity of ACPC should be continued and be ready for coming to clinical trials.

Indefinite effects of different source and constituent of ACPC

ACPC could be got from two different sources: by extraction from vegetables or fruits of rich endogenous ACPC and by artificial chemical synthesis. Despite their molecular formula and chemical structure are the same, it is still unsure, if the function and efficacy have no difference between the two sources of ACPC. Corresponding comparative studies are not found, which studied whether there was differences or not about effects or efficacy on animals with use of ACPC from two different sources namely natural extraction and chemical synthesis. In the previous investigations, it was not reported from which source that ACPC was used in the experiments and the discrepancy of influences on animals or humans. Therefore, this finding suggests that not only the effects and efficacy of ACPC, but also the dose and safety from the two sources should be further investigated in animals. Which one is better or they are identical has great meaning to the mass production of ACPC. Such studies

would also provide evidences for clinical trials.

Furthermore, ACPC is a potent and selective ligand for the glycine modulatory site complex related NMDAR (Marvizón, Lewin, and Skolnick, 1989). However, it was not exactly known that the mechanism of the effects of ACPC is through single ACPC itself alone, or the ACPC complex of the glycine modulatory site. Scientists have not got the ACPC complex substance so far and not known the exact structure of the ACPC complex. So ACPC complex certainly could not be prepared as well. Perhaps effect of ACPC complex is greatly more than simple ACPC. It is necessary that we firstly derive the ACPC complex from organism, then analyze its structure and prepare it. Finally its bioactivities should be determined by sending it back to the organism.

Fortunately, the real dose of ACPC for human would not be so large in practice. Vegetables or fruits containing very low content of ACPC are of obvious advantages for human in cerebro- and cardiovascular system diseases. In silk worm, 0.1–1 μg ACPC/100 g mulberry leaf will play a great role of the bioactivity in increasing silk protein yield by 30% (Zhang *et al*, 1990). The reason of why the dose difference of endogenous ACPC and exogenous ACPC is so great is still unclear. Maybe the key consists in the synergism of endogenous ACPC and other active constituents in organism.

Prospect and proposal of future studies

Finding some good ways to solve these problems is strongly suggested in this thesis. In order to get rid of the limitations of previous studies, further large clinical trials or epidemiological studies would be demanded to identify these effects of ACPC in humans. The dose and economic problems in clinical trials must be firstly solved. One of the solutions would be popularization of novel synthetic routes of ACPC (Fig. 2 and Fig. 3) for mass production of ACPC with low cost. Moreover, mass extracting of ACPC derived from vegetables or fruits of rich endogenous ACPC promoted by small content of exogenous ACPC is maybe another way.

According to the effects of ACPC on people in medical aspect, following studies are suggested to be further carried on.

Developed into neuroprotective agent

As the neuroprotective effects of ACPC become well known, it is hoped to develop ACPC as a new type neuroprotective agents, which should prevent damage to the brain or spinal cord from ischemia, stroke, convulsions, or trauma. Some conventional neuro-protective drugs must be administered before the event, but others may be effective for some time after. These drugs act by a variety of mechanisms, but often directly or indirectly minimize the damage produced by endogenous excitatory amino acids, of which glutamate induced excitatory toxicity occurs most frequently. Nimodipine is one of the most widely used clinical drugs of neuroprotection. It could alleviate the brain injury caused by ischemia and directly protect the neurons. Therefore, in this work Nimodipine was used as the positive control to compare with ACPC. The results indicated that ACPC significantly reduced the brain damage after ischemic insult *in vivo* and attenuated glutamate-induced neurotoxicity such as apoptosis of cerebral granular neurons *in vitro* ($P < 0.001$, 0.01).

However, these available agents have some adverse side effects such as psychotomimetic properties in humans and disturbance in breath and blood pressure, etc. (Olney *et al*, 1991). ACPC was reported to be an uncompetitive antagonist on the glycine-site at NMDAR. The activation of NMDAR is a complicated process. The coordination of glu-site and gly-site is required, of which any single transmitter can not open the ion channel. Glycine is a regulator of NMDAR. The antagonist, which acts on the gly-site, has the superiority of partial agonist. So it could theoretically avoid the adverse reactions of those entire antagonists in different degree. ACPC mostly acts on the position of abnormal or excessive accumulation of glutamate and has minor influences on normal neurotransmission. ACPC not only effectively inhibits the neurotoxicity induced by some neurotransmitter with high concen-tration in certain area of brain, but also at the same time maintains the normal cerebral neurotransmission in other areas.

In consequence, ACPC has no those adverse or toxic side effects, which happened in current excitatory amino acids antagonists. However, further clinical researches on ACPC of development into a drug are required and should be warranted, although in the USA it has entered into clinical trials Phase Ⅰ as a cerebral nerves protective drug since 1995. But the drug still stays in the same process so far. Further clinical trials phase Ⅱ would be necessary. Continued re-evaluation

and sharing of information derived from the laboratory bench or the patient's bedside should eventually lead to effective neuroprotection in acute stroke. Experimental data should be carefully studied to improve the quality of agents coming to clinical trials and to design trial phasing that effectively determines drug safety and efficacy (Degraba and Pettigrew, 2000). ACPC is indeed an excellent neuroprotective agent of the central nervous system and promisingly become to a new medicine for the treatment and prevention of neural degeneration diseases.

Developed into anti-infective agent

Nowadays, although infectious diseases are no longer the primary cause of death, and a large number of agents used for the prevention or treatment of bacterial infections are available, the emergence of resistant bacterial organisms poses a serious problem in infectious diseases. This problem occurs, in particular, in the hospital environment, in which outbreaks of highly resistant strains of e.g. *Staphylococcus aureus*, Klebsiella, among many others, pose a constant threat (Levy, 1998). This problem even extends to the larger community. A well known example is penicillin-resistant *Streptococcus pneumoniae* (Simberkoff, 1994).

The inevitability of resistance on account of microbial mutations results in the ongoing need for the development of anti-bacterial therapeutic agents. ACPC, one of the non-protein amino acids, has been demonstrated as an anti-infective agent for treating infections caused by bacteria including gram positive bacteria, such as *S. aureus*, and gram negative bacteria, such as *E. coli* (Rubenstein, 2001); viruses such as Newcastle, coronavirus; helmithic parasites and protozoan such as coccidiosis (Zhu, 2005). Among them, three kinds of bacteria were first determined in 2005 by Zhejiang Medical Academy of Science, China. They are *S. aureus*, *E. coli*, and *C. albicans*.

The minimal impact on the host is an excellent feature of ACPC and anti-infective non-protein amino acids. Preferred is ACPC, which is found in plants and in principle, is non-toxic to humans and commonly eaten by humans. As antibiotics are currently abused and the resistance becomes increasingly serious, the need for the development of compounds which are useful as anti-infective agents with fewest and mildest side effects on the host becomes more urgent. ACPC as the novel antibiotics of new mechanism is of important meaning of strategy.

Prevention and control of hypertension

Since ACPC has shown its positive protection of brain and nervous system, currently, the research focus is more on the prevention and therapeutic action for cerebral ischemia and the related cerebro- and cardiovascular system diseases. The recent study by Gao *et al* (2007) reported that ACPC has important cardiovascular effects, precisely hypotensive and antioxidant effects. Their finding provided an evidence of the normalization by ACPC of blood pressure elevation and mortality of stroke. Zhu (2005; 2007) also revealed in his invention the effects of ACPC on anti-hypertension, prevention, and treatment of stroke. Nevertheless, more studies on the effects of ACPC on hypertension were not found. It still remains a new function of ACPC, which was but currently reported. For that, lots of further clinical trials or public health studies should be made to determine the meaningful action on humans, which makes good sense at the present situation with high incidence and prevalence of hypertension, but no suitable drugs for it without adverse side-effects and dependence.

Overweight and hypertension represent the most important risk factors for the formation of cardio-vascular diseases. An overly high blood pressure is one of the most frequent reasons for overweight and hypertension. Hypertension is internationally defined as the increase of blood pressure in at least 140 mmHg by systolic pressure and/or 90 mmHg by diastolic pressure, which are measured many times and controlled in silence (definition of WHO). Almost every third adult in Germany suffered from hypertension in the last years. In China, the morbidity was recently measured to be over 20 percent in people from the age of 15 years and up. Altogether, there were 160 million patients with elevated blood pressure in China. The meaning of the arterial hypertonic as an essential risk factor for cardio-vascular illnesses is doubtless in numerous examina-tions. According to the analysis of MacMahon, an increase by 5 mmHg of the diastolic blood pressure creates a higher risk by 20% in each case (MacMahon, 1990).

Since the 20th Century, hypertension counts as one of the most frequent chronic disturbances of the heart cycle system with serious consequences for the

concerned people, as well as the whole health system in the industrial countries and also recently in the developing countries. Although medical procedures for control of an elevated blood pressure have existed for a long time, their benefits and side effects of poor compliance still remain in question. As a result, since the sixties, different behavioral treatments have been developed with the aim to be used as supplements to the drug therapy or even replace it (Fleisch, 1996).

There are versatile influential factors that can lead to high blood pressure, with what the most important are genetic reason, little movement and faulty nutrition (Kunz *et al*, 2003). Nutrition is in this occasion of particular importance for the hypertension. The second important column with the lowering of the blood pressure after medications is the nutritional changeover. A public health study is advised, which should deal with ACPC for itself with the rise of the influence of amino acid-containing nutrition on the hypertension, specifically with ACPC.

During the first American DASH-Study (Dietary Approaches to Stop Hypertension) in 1997, the positive influence of fruit and vegetable, low-fat as well as fat-modified nutrition (DASH-Diet) was proved on the blood pressure (Appel, Moore, and Obrazanek, 1997). However, it remained unclear at that time, how the influence of fruit and vegetables works. A current epidemiological research in London in 2006 proved that vegetable protein has a reverse connection with blood pressure. Vegetable protein intake something inversely related to blood pressure. This finding is consistent with recommendations that a diet high in vegetable products be part of a healthy lifestyle for prevention of high blood pressure and related diseases (Elliott *et al*, 2006). The mechanism of this relationship between the active substances is otherwise not known, yet. Prof. ZHU Xu-xiang discovered through countless experiments and attempts after over 20 years that ACPC is exactly the active substance of the plant protein for hypertension (Zhu, 2005).

Therefore, in the future, ACPC might be a food therapy for circle system diseases. Along with the development of society, the cerebrovascular and cardiovascular diseases become increasingly popularization in younger people, leading to be the first killer of human health. Moreover, survivors usually because of hemiplegia and aphasia etc. sequelas lose their work ability and even become totally depended on others in daily live, which is becoming a serious social problem. The outbreak mechanism of the cerebrovascular and cardiovascular diseases is most relevant to a high blood pressure.

Hypertension is either an independent disease, or a risk factor for stroke, coronary heart disease and kidney degradation. If the average in diastolic blood pressure in patients with 3 mmHg drops, the risk of stroke will drop by 32%, the risk of coronary heart disease will drop by 19% and the incidence of other complications will be also geometrically reduced. High incidence and prevalence, high morbidity and mortality, high recurrence rate as well as more complications are normal phenomena of hypertension. High blood pressure is caused, besides by innate genetic factors, mainly by the acquired live habits, in particular, caused by the structure of foods, such as high-protein, high fat, high sodium, low fiber. Nowadays, at home and abroad, there is no effective way to prevent hypertension and no specific drug or treatment. Patients must take medicine to reduce blood pressure every day over years, but can not bear over the financial burden and suffering.

ACPC, a natural active composition in fruits and vegetables, can significantly control the blood pressure, anti-thrombosis, prevention and treatment of stroke and the cerebral neuroprotective effects. It protects effectively the heart, brain, and kidney against injury without toxic side effects and is on the safe side. Consequently, adopting the method of food intervention, precise treatment with the prescription of vegetables and consuming fruits that are rich in ACPC can prevent the hypertension and thereby increase the public health level, dismiss public pain and sufferings, alleviate the medical treatment costs and reduce economic losses. At the same time, the economic and social development in harmony could be promoted.

In order to realize the prospect of “food as drug” with ACPC through which its effects could be applied in humans, epidemiological or public health studies or DASH surveys are suggested to carry out in a community of residence with high prevalence of hypertension. One of them would be the prevention and treatment of hypertension with food intervention. The study should investigate whether the blood pressure by the administration of ACPC-rich fruits and vegetables

will decrease and thereby hypertension would be prevented and treated. Three plants are mainly used: tomato, kiwi fruit and celery. The aim of the study is to identify the effectiveness of ACPC on hypertension in the population in conjunction with an epidemiological study, which carries a nutritional intervention. The aim of nutrition intervention is to reduce the prevalence of hypertension (controlled and uncontrolled hypertension) in the population through primary preventive measures, namely the change of diet and improving the health level of the population.

People have taken a low content of ACPC in fruits and vegetables, but the intake of pure ACPC is required to control hypertension. This means that pure ACPC could greatly effectively prevent and treat hypertension. It may be helpful for the clinical study to develop ACPC into a natural drug without adverse reaction.

This is also the final purpose of the present work that the product with the active substance of ACPC could be applied in the clinical treatment and also be taken as a nutrient including in the diet in everyday life. Then, in the near future, it should be successfully marketed all over the world to benefit human beings and result in an improvement of the health level of the population.

Conclusion

The intention of this thesis is to provide a comprehensive overview of effects of ACPC reported up to now and to display the current research situation of it. ACPC is a natural non-protein amino acid existing originally in plants, and is of unique bioactivities. The regulating influences of ACPC on plants are well known. ACPC not only regulates plant growth and development, but also protects plants against infestation by virus, bacteria and insect, and thereby protects agriculture from disastrousness.

A variety of effects on animals is the focal point of this work. In summary, ACPC was reported to have altogether six general effects on animals.

First, ACPC promotes animal growth and improves reproductive quality.

Second, ACPC protects the brain and nervous system: neuroprotection against excitatory toxicity, and brain injury caused by ischemia or hypoxia, anti-depressant and anxiolytic actions, anti-convulsion and -seizures and control of epilepsy, facilitating spatial learning and memory as well as reducing alcohol consumption.

Third, ACPC affects the cerebro- and cardiovascular system and can be used to control hypertension, prevent and treat stroke and heart infarction.

Fourth, ACPC protects internal organs (e.g. kidney and liver) and their cells from hypoxic injury.

Fifth, ACPC acts as an anti-infection against virus, bacteria and parasitic diseases. For example, *S. aureus*, *E. coli*, and Candida *albicans* are definite to be effectively sterilized by ACPC.

Sixth, ACPC is an anti-radiation of mobile phones, microwaves, screens and so on.

Moreover, ACPC has some new pharmacological effects on anti-high blood pressure, anti-thrombosis and prevention and treatment of stroke. It is of great significance to hypertension, cardio-cerebral thrombosis, and stroke, effective protection to the heart, kidneys and brain with non-toxic side effects.

ACPC has been demonstrated as anti-infective agent for treating infections caused by bacteria including gram positive bacteria, such as *S. aureus*; gram negative bacteria, such as *E. coli* (Rubenstein, 2001); viruses such as Newcastle, coronavirus; helmithic parasites and protozoan such as coccidiosis (Zhu, 2005).

Although the present work indicated a few bioactivities and pharmaceutical effects of ACPC, research on its physiological property and mechanism of effects is still imperfect. Only a few articles mentioned experiments on animals that explored other actions of ACPC besides the neuroprotective effects. Further activities of ACPC still remain a mystery. Thus, studies on the effects of ACPC with animal experiment should be continued to prepare it for clinical trials as well.

Another limitation of reviewed studies is the unknown effects of ACPC on the human body. Previous researches included mostly small, experimental studies with animal models or cell cultures. No large clinical trials or database was available to demonstrate the efficacy of ACPC on the population level. Consequently, to determine the effects of ACPC in humans, it is strongly suggested that further large clinical trials or epidemiological studies should be designed and continually carried out.

Fortunately, vegetables and fruits containing

ACPC are of obvious advantages for humans with cerebro- and cardiovascular system diseases, especially for hypertension. That means, people could prevent and control these diseases by vegetables and fruits. Therefore, food intervention of vegetables and fruits rich in ACPC could prevent and treat hypertension and stroke, and thereby increase the public health level, which would make great sense in the present situation with the high incidence and prevalence of hypertension, but no suitable drugs without adverse side-effects and dependence available.

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References

Abeles FB, 1973. Ethylene in Plant Biology. Academic Press, New York.

Adams DO, Yang SF, 1979. Ethylene biosynthesis: Identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. *Proc Natl Acad Sci* 76(1): 170-174.

Appel LJ, Moore TJ, Obrazanek E, 1997. A clinical trial of the effects of dietary patterns on blood pressure. *N Engl J Med* 336: 1117-1124.

Bederson JB, Pitts LH, Tsuji M, Nishimura MC, Davis RL, Bartkowski H, 1986. Rat middle cerebral artery occlusion: Evaluation of the model and development of a neurologic examination. *Stroke* 17(3): 472-476.

Boje KM, Wong G, Skolnick P, 1993. Desensitization of the NMDA receptor complex by glycinergic ligands in cerebellar granule cell cultures. *Brain Res* 603: 207-214.

Bousquet JF, Thimann KV, 1984. Lipid peroxidation forms ethylene from 1-aminocyclopropane-1-carboxylic acid and may operate in leaf senescence. *Proc Nati Acad Sci* 81:1724-1727.

Bovetto S, Boyer PA, Skolnick P, Fossom LH, 1997. Chronic administration of a glycine partial agonist alters the expression of *N*-methyl-*D*-aspartate receptor subunit mRNAs. *J Pharmacol Exp Thera* 283(3): 1503-1508.

Brecht M, de Groot H, 1994. Protection from hypoxic injury in cultured hepatocytes by glycine, alanine, and serine. *Amino Acids* 6: 25-35.

Burroughs LF, 1957. 1-Aminocyclopropane-1-carboxylic acid: A new amino-acid in perry pears and cider apples. *Nature* 179(4555): 360-361.

Burroughs LF, 1960. The free amino acid of certain British fruits. *J Sci Food Agric* 11: 14-18.

Butler GW, Peterson PJ, 1967. Uptake and metabolism of inorganic forms of selenium-75 by *Spirodela oligorrhiza*. *Aust J Biol Sci* 20: 77-86.

Cameron AC, Fenton CAL, Yu Y, Adams DO, Yang SF, 1979. Increased production of ethylene by plant tissues treated with 1-amino-cyclopropane-1-carboxylic acid. *Hortscience* 14(2): 178-180.

Castro-Puyana M, Crego AL, Marina ML, García-Ruiz C, 2007. CE methods for the determination of non-protein amino acids in foods. *Electrophoresis* 28(22): 4031-4045.

Cherkofsky SC, 1995. 1-aminocyclopropanecarboxylic acid: Mouse to man interspecies pharmacokinetic comparisons and allometric relationships, *J Pharm Sci* 84(10): 1231-1235.

Choi DW, 1988. Glutamate neurotoxicity and diseases of the nervous system. *Neuron* 1(8): 623-634.

Cowie DB, Cohen GN, 1957. Biosynthesis by *Escherichia coli* of active altered proteins containing selenium instead of sulfur. *Biochim Biophys Acta* 26(2): 252-261.

DeGraba TJ, Pettigrew LC, 2000. Why do neuroprotective drugs work in animals but not humans? *Neurol Clin* 18(2): 475-493.

Ding CM, Zhu XX, Zhang ZM, 2004. Test report of ACPC feeding table poultry. *China Anim Husb Vet Med* 31(2): 43.

Dirnagl U, Iadecola C, Moskowitz MA, 1999. Pathobiology of ischaemic stroke: An integrated view. *Trends Neurosci* 22(9): 391-397.

Elliott P, Stamler J, Dyer AR, Appel L, Dennis B, Kesteloot H, Ueshima H, Okayama A, Chan Q, Garside DB, Zhou B, 2006. Association between protein intake and blood pressure: The INTERMAP Study. *Arch Intern Med* 166(1): 79-87.

Faden AI, Jacobs TP, 1984. Dynorphin-related peptides cause motor dysfunction in the rat through a non-opiate mechanism, *Br J Pharmacol* 81: 271.

Fleisch JM, 1996. Möglichkeiten der Blutdrucksenkung durch operantes Konditionieren unter Einsatz einer neuen Biofeedback-Anordnung. Dissertation der Medizinischen Fakultät der Universität Tübingen.

Fossom LH, Basile AS, Skolnick P, 1995. Sustained exposure to 1-aminocyclopropanecarboxylic acid, a glycine partial agonist, alters *N*-methyl-*D*-aspartate receptor function and subunit composition. *Mol Pharmacol* 48(6): 981-987.

Fossom LH, Von Lubitz DK, Lin RC, Skolnick P, 1995. Neuroprotective actions of 1-aminocyclopropanecarboxylic acid (ACPC): A partial agonist at strychnine-insensitive glycine sites. *Neurol Res* 17(4): 265-269.

Gao M, Kondo F, Murakami T, Xu JW, Ma N, Zhu XX, Mori K, Ishida T, 2007. 1-Aminocyclopropanecarboxylic acid, an antagonist of *N*-methyl-*D*-aspartate receptors, causes hypotensive and antioxidant effects with upregulation of heme oxygenase-1 in stroke-prone spontaneously hypertensive rats. *Hypertens Res* 30(3): 249-257.

Grant KA, Valverius P, Hudspith M, Tabakoff B, 1990. Ethanol withdrawal seizures and the NMDA receptor complex. *Eur J Pharmacol* 176(3): 289-296.

Haque MM, Nasreen SA, 2008. Epidemiological study of risk factors of stroke and its immediate consequence. *Mymensingh Med J* 17(2): 124-128.

Herman BH, Goldstein A, 1985. Antinociception and paralysis induced by intrathecal dynorphin A. *J Pharmacol Exp Ther* 232(1): 27-32.

Heyman S, Spokes K, Rosen S, Epstein FH, 1992. Mechanism of glycine protection in hypoxic injury: Anologies with glycine receptor. *Kidney Int* 42(1): 41-45.

Howell SE, Miller SR, McCallister JD, Cherkofsky SC, Patrick KS, 1995. Gas chromatographic-mass spectrometric determination of urinary 1-aminocyclopropanecarboxylic acid in mice using a deuterated internal standard. *J Chromatogr B Biomed Appl* 663(1): 148-152.

Jia M, Dong Z, Zhu Y, Guan YB, 2006. Molecular targets of Chinese used in treatment of stroke. *Chin Tradit Herb Drugs* 37(12): 1908-1911.

Kolesnikov YA, Maccechini ML, Pasternak GW, 1994. 1-Amino-cyclopropane carboxylic acid (ACPC) prevents mu and delta opioid tolerance. *Life Sci* 55(18): 1393-1398.

Kunz C, Linn T, Gortner L, Bretzel RG, 2003. Ernährungsprobleme bei Kindern und Erwachsenen. Spiegel der Forschung 20. Jg/Nr.1/2; 19ff.

Layer RT, Bland LR, Skolnick P, 1993. MK-801, but not drugs acting at strychnine-insensitive glycine receptors, attenuate methamphe-tamine nigrostriatal toxicity. *Brain Res* 625(1): 38-44.

Lea PJ, Norris RD, 1976. The use of amino acid analogues in studies of plant metabolism. *Phytochemistry* 15: 585-595.

Levy SB, 1998. Multidrug resistance-a sign of the times. *N Engl J Med* 338(19): 1376-1378.

Liang ZH, Wei EQ, Zhu CY, Zhang SH, Xu HM, 2003. An improved imaging analysis for quantitative measurement of brain slice volume. *J Zhejiang Univ (Med Sci)* 32(6): 497-501.

Lin JC, Tsao WL, Wang Y, 1995. Cardiovascular effects of NMDA in the RVLM of spontaneously hypertensive rats. *Brain Res Bull* 37(3): 289-294.

Lin Y, Fossom LH, Skolnick P, Long JB, 1998. Sustained exposure to a glycine receptor partial agonist differentially alters NMDA receptor agonist and antagonist potencies in cultured spinal cord neurons. *Eur J Pharmacol* 356: 255-260.

Long JB, Petras JM, Mobley WC, Holaday JW, 1988. Neurological dysfunction after intrathecal injection of dynorphin A (1-13) in the rat. II. Non-opioid mechanisms mediate loss of motor, sensory, and autonomic function. *J Pharmacol Exp Ther* 246(3): 1167-1174.

Long JB, Rigamonti DD, Oleshansky MA, Wingfield CP, Martinez-Arizala A, 1994. Dynorphin A-reduced rat spinal cord injury: Evidence for excitatory amino acid involvement in a pharmacological model of ischemic spinal cord injury. *J Pharmacol Exp Ther* 269(1): 358-366.

Long JB, Skolnick P, 1994. 1-Aminocyclopropanecarboxylic acid protects against dynorphin A-induced spinal injury. *Eur J Pharmacol* 261(3): 295-301.

Longa EZ, Weinstein PR, Carlson S, Cummins R, 1989. Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke* 20(1): 84-91.

Lopes T, Neubauer P, Boje KM, 1997. Chronic administration of NMDA glycine partial agonists induces tolerance in the Porsolt swim test. *Pharmacol Biochem Behav* 58(4): 1059-1064.

Lundy EF, Solik BS, Frank RS, Lacy PS, Combs DJ, Zelenock GB, D'Alecy LG, 1986. Morphometric evaluation of brain infarcts in rats and gerbils. *J Pharmacol Methods* 16(3): 201-214.

Lürssen K, Naumann K, Schröder R, 1979. 1-Aminocyclo-propane-1-carboxylic acid — An intermediate of ethylene biosynthesis in higher plants. *Z Pflanzenphysiol* 92: 285-294.

MacMahon S, Peto R, Cutler J, Collins R, Sorlie P, Neaton J, Abbott R, Godwin J, Dyer A, Stammler J, 1990. Blood pressure, stroke and coronary heart disease. Part 1: Prolonged differences in blood pressure. Prospective observational studies corrected for the regression dilution bias. *Lancet* 335: 765.

Manev H, Costa E, Wroblewski JT, Guidotti A, 1990. Abusive stimulation of excitatory amino acid receptors: A strategy to limit neurotoxicity. *FASEB J* 4(10): 2789-2797.

Marvizón JC, Lewin AH, Skolnick P, 1989. 1-Aminocyclopropane carboxylic acid: A potent and selective ligand for the glycine modulatory site of the *N*-methyl-*D*-aspartate receptor complex. *J Neurochem* 52(3): 992-994.

Meyer BU, Schneider W, Elstner EF, 1992. Peroxide-dependent amino acid oxidation and chemiluminescence catalysed by magnesium-pyridoxal phosphate-glutamate complex. *Biochem Pharmacol* 44(3): 505-508.

Mills E, Minson J, Drolet G, Chalmers J, 1990. Effect of intrathecal amino acid receptor antagonists on basal blood pressure and pressor responses to brainstem stimulation in normotensive and hypertensive rats. *J Cardiovasc Pharmacol* 15(6): 877-883.

Muir KW, Lees KR, 1995. Clinical experience with excitatory amino acid antagonist drugs. *Stroke* 26(3): 503-513.

Nagasawa H, 1995. Effect of ACC, a component of *Coprinus comafus*, on fertility of male mice. *J Meiji Daigaku Nogakubu Kenkyu Hokoku* 102: 9-15.

Nahum-Levy R, Fossom LH, Skolnick P, Benveniste M, 1999. Putative partial agonist 1-aminocyclopropanecarboxylic acid acts concurrently as a glycine-site agonist and a glutamate-site antagonist at *N*-methyl-*D*-aspartate receptors. *Mol Pharmacol* 56(6): 1207-1218.

Nowak G, Li Y, Paul IA, 2000. Chronic glycine treatment desensitizes the behavioral response to 1-aminocyclopropanecarboxylic acid (ACPC), a partial agonist at the strychnine-insensitive glycine site of the NMDA receptor complex. *J Neural Transm* 107(2): 123-131.

Olney JW, Labruyere J, Wang G, Wozniak DF, Price MT, Sesma MA, 1991. NMDA antagonist neurotoxicity: Mechanism and prevention. *Science* 254(5037): 1515-1518.

Papp M, Gruca P, Willner P, 2002. Selective blockade of drug-induced place preference conditioning by ACPC, a functional NDMA-receptor antagonist. *Neuropsychopharmacology* 27(5): 727-743.

Papp M, Moryl E, 1996. Antidepressant-like effects of 1-amino-cyclopropanecarboxylic acid and *D*-cycloserine in an animal model of depression. *Eur J Pharmacol* 316(2-3): 145-151.

Patat A, Molinier P, Hergueta T, Brohier S, Zieleniuk I, Danjou P, Warot D, Puech A, 1994. Lack of amnestic, psychotomimetic or impairing effect on psychomotor performance of eliprodil, a new NMDA antagonist. *Int Clin Psychopharmacol* 9(3): 155-162.

Peterson SL, 1995. Infusion of NMDA antagonists into the nucleus reticularis pontis oralis inhibits the maximal electroshock seizure response. *Brain Res* 702(1-2): 101-109.

Peterson SL, Purvis RS, Griffith JW, 2004. Differential neuro-protective effects of the NMDA receptor-associated glycine site partial agonists 1-aminocyclopropanecarboxylic acid (ACPC) and *D*-cycloserine in lithium-pilocarpine status epilepticus. *Neuro-toxicology* 25(5): 835-847.

Popik P, Rygielaka Z, 1999. A partial agonist at strychnine-insensitive glycine sites facilitates spatial learning in aged rats. *J Physiol Pharmacol* 50(1): 139-151.

Priestley T, Horne AL, McKernan RM, Kemp JA, 1990. The effect of NMDA receptor glycine site antagonists on hypoxia-induced neurodegeneration of rat cortical cell cultures. *Brain Res* 531(1-2): 183-188.

Przegaliński E, Tatarczyńska E, Dereń-Wesołek A, Chojnacka-Wójcik E, 1996. Anticonflict effects of a competitive NMDA receptor antagonist and a partial agonist at strychnine-insensitive

glycine receptors. *J Pharmacol-Biochem-Behav* 54(1): 73-77.

Przegaliński E, Tatarczyńska E, Dereń-Wesołek A, Chojnacka-Wojcik E, 1997. Antidepressant-like effects of a partial agonist at strychnine-insensitive glycine receptors and a competitive NMDA receptor antagonist. *J Neuropharmacol* 36(1): 31-37.

Przegaliński E, Tatarczyńska E, Kłodzińska A, Chojnacka-Wójcik E, 1999. Tolerance to anxiolytic- and antidepressant-like effects of a partial agonist of glycineB receptors. *Pharmacol Biochem Behav* 64(3): 461-466.

Rogawski MA, 2000. Low affinity channel blocking (uncompetitive) NMDA receptor antagonists as therapeutic agents--toward an understanding of their favorable tolerability. *Amino Acids* 19(1): 133-149.

Rubenstein E, 2001. Methods for the use of nonprotein amino acids as therapeutic agents. United States Patent 6191168. Inventors: Rubenstein, Edward (5 Waverly Pl., Hillsborough, CA, 94010). Application Number: 09/324181. Publication Date: 02.20.2001.

Schneider W, 1987. Der hypocholesterinämische und antiathero-sklerotische Einfluß von Magnesium-Pyridoxal-5'-phosphat-Glutamat im Serum, in der Leber und der Aorta hyper-cholesterinämischer Ratten und Kaninchen", Dissertation an der Johannes Gutenberg-Universität, Mainz.

Schroeder R, Luerssen K, 1980. Mittel zur Regulierung des Pflanzenwachstums. Ger Offen 2906507, CA94: P83668p.

Simberkoff, MS, 1994. Drug-resistant pneumococcal infections in the United States. A problem for clinicians, laboratories, and public health. *J Am Med Assoc* 271(23): 1875-1876.

Skolnick P, Marvizón JC, Jackson BW, Monn JA, Rice KC, Lewin AH, 1989. Blockade of *N*-methyl-*D*-aspartate induced convulsions by 1-aminocyclopropanecarboxylates. *Life Sci* 45(18): 1647-1655.

Skolnick P, Miller R, Young A, Boje K, Trullas R, 1992. Chronic treatment with 1-aminocyclopropanecarboxylic acid desensitizes behavioral responses to compounds acting at the *N*-methyl-*D*-aspartate receptor complex. *Psychopharmacol* 107(4): 489-496.

Stevens CW, Yaksh TL, 1986. Dynorphin A and related peptides administered intrathecally in the rat: A search for putative kappa opiate receptor activity. *J Pharmacol Exp Ther* 238(3): 833-838.

Stromberg MF, Volpicelli JR, O'Brien CP, Mackler SA, 1999. The NMDA receptor partial agonist, 1-aminocyclopropanecarboxylic acid (ACPC), reduces ethanol consumption in the rat. *Pharmacol Biochem Behav* 64(3): 585-590.

Swain T, 1977. Secondary compounds as protective agents. *Annu Rev Plant Physiol* 28: 479-501.

Trullas R, Folio T, Young A, Miller R, Boje K, Skolnick P, 1991. 1-amino-cyclopropanecarboxylates exhibit antidepressant and anxiolytic actions in animal models. *Eur J Pharmacol* 203(3): 379-385.

Trullas R, Skolnick P, 1990. Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. *Eur J Pharmacol* 185(1): 1-10.

Vähätalo ML, Virtanen AI, 1957. Cyclic α-aminocarboxylic acid in berries of cowberry. *Acta Chem* 11: 741-743.

Viu E, Zapata A, Capdevila J, Skolnick P, Trullas R, 2000. Glycine(B) receptor antagonists and partial agonists prevent memory deficits in inhibitory avoidance learning. *Neurobiol Learn Mem* 74(2): 146-160.

Von Lubitz DK, Lin RC, McKenzie RJ, Devlin TM, McCabe RT, Skolnick P, 1992. A novel treatment of global cerebral ischemia with a glycine partial agonist. *Eur J Pharmacol* 219(1): 153-158.

Wang FR, Jiang YS, Xiao WW, Zhang SM, 2003. Mice focal cerebral ischemia-reperfusion model established by internal carotid artery suture method. *Stroke Nerv Dis* 10(2): 112-114.

Weinberg JM, Venkatachalam MA, Roeser NF, Davis JA, Varani J, Johnson KJ, 1991. Amino acid protection of cultured kidney tubule cells against calcium ionophore-induced lethal cell injury. *Lab Invest* 65(6): 671-678.

Yang SF, 1984. The formation of ethylene from l-aminocyclo-propane-1-carboxylic acid. In: R.Y. Fuchs and E. Chalutz (Eds.). Ethylene: Biochemical, Physiological and Applied Aspects. Martinus Nijhoff/Dr. W. Junlc Publishers. The Netherlands, 1-10.

Yang SF, 1998. A bit in the research on ethylene biosynthesis. *Sci Knowledge* 47: 1-6.

Yang SF, Hoffman NE, 1984. Ethylene biosynthesis and its regulation in higher plants. *Annu Rev Plant Physiol* 35: 155-189.

Yao JF, Zhang ZM, Xu GP, Xu FQ, Zhou HC, Wang XH, Deng B, 2001. Feeding test report of ACC on poultry. Zhejiang Agricultural Academy of Sciences, Department of Animal Husbandry and Veterinary Medicine.

Ye CL, Li BH, 1993. Protective effects of clonidine on cerebral ischemia in mice and cats. *Chin J Pharmacol Toxical* 7(1): 42-44.

Yu YB, Adams DO, Yang SF, 1979. 1-Aminocyclopropanecarboxylate Synthase, a Key Enzyme in Ethylene Biosynthesis. *Arch Biochem Biophysics* 198 (1): 280-286.

Zapata A, Capdevila JL, Viu E, Trullas R, 1996. 1-Aminocyclo-propane-carboxylic acid reduces NMDA-induced hippocampal neurodegeneration *in vivo*. *Neuroreport* 7(2): 397-400.

Zhang J, Xu JL, Zhu XX, 1990. Nonprotein amino acid ACC regulates and controls the growing development of silkworm. *J Bull Sericult* 21(4): 30-31.

Zhao ZR, 2005. Protection of ACC on brain neurological impairment induced by ischemia. Master Dissertation of Zhengjiang Academy of Medical Sciences.

Zhao ZR, Zhu QW, Zhu XX, 2005. Protection of 1-aminocyclo-propanecarboxylic acid against brain neurological impairment by ischemia. *Chin Tradit Herb Drugs* 36(10): 1523-1527.

Zhu XX, 1998. *The Regulating Effects of ACC on the Rice*. Advances in plant growth substance and herbicide research and application (I). China Agricultural Press, Beijing, 61-68.

Zhu XX, 2003. New feed additive ACPC. New Feed Certificate No.[2003]02 of The Agriculcure Departmemnt of the People's Republic of China.

Zhu XX, 2005. 1-Aminocylopropanecarboxylic Acid (ACC) on Cardiocerebrovascular Diseases. Bulletin of Invention Patent I of China, 2005, 21 (26), 57: 200410010516.0 (CN 1631361A).

Zhu XX, 2007. Application of ACPC in preparation of medicine against cardiovascular diseases. China National Patent of Invention ZL 200410010516.0.

Zhu XX, Xu JL, 1991. Physiological actions of nonprotein amino acid ACC of animals. *Bull Sci Technol* 7(4): 237.